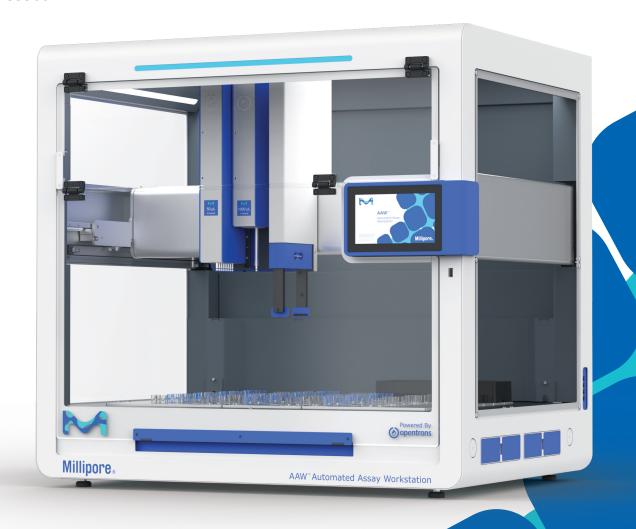


User Guide

AAW[™] Automated Assay Workstation Assay Ready

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Millipore_®

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Introduction

Supporting Lab of the Future

MilliporeSigma, the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany, and Opentrons Labworks, Inc., a leader in lab automation and accessible robotics, have partnered to automate assay kits on the custom robot, the AAW™ Automated Assay Workstation - Assay Ready.

Scientists and engineers have collaborated to develop and verify platform workflows utilizing the broad offering of automation-enabled assays. Together, the AAW^{TM} Workstation and automation-enabled assays will deliver increased consistency and higher throughput by reducing manual processing and repetitive tasks with a user-friendly robotic system.

AAW™ Automated Assay Workstation - Assay Ready

AAW™ Automated Assay Workstation includes hardware accessories needed for a variety of applications. All workstation accessories are modular. To change applications, add or swap in other hardware and compatible consumables.

Components Included

- AAW[™] Automated Assay Workstation
- AAW[™] 1-Channel Pipette, 1000 μL
- AAW™ 8-Channel Pipette, 1000 µL
- AAW[™] Flex Gripper
- Opentrons Flex[™] Magnetic Block
- Opentrons Flex[™] Deck Expansion (4)
- Opentrons Flex™ Heater-Shaker, with 4 adapters:
 - Universal Flat Adapter
 - Flat Bottom Plate Adapter
 - PCR Adapter
 - Deep Well Adapter

Millipore® Protocol Library

The AAW™ Workstation provides access to the Millipore® Protocol Library, which contains a growing list of verified protocols to easily automate SMC® assays, Duolink® assays, and Recombinant Protein expression screening, as examples. Users will also have access to the vast Opentrons® open-source library, and accessibility and compatibility with generative AI tools.



Feature Details

Feature	Description	
Pipette throughput	The Opentrons® pipettes have 1-, 8-, or 96-channels. The 96-channel pipette works on 96 times as many wells as the single channel pipette, or 12 times as many as the 8-channel pipette.	
Pipette and tip capacities	The AAW TM pipettes have larger volume ranges (1–50 μ L, 5–1000 μ L) and can all work with any size of Flex pipette tips.	
AAW™ Flex Gripper	The AAW™ Flex Gripper picks up and moves labware around the deck automatically, without user intervention. This enables more complex workflows within a single protocol run.	
Automated calibration	Positional calibration of the pipettes and the gripper is fully automated. Press one button, and the instrument will move to precision-machined points on the deck to determine its exact position, saving that data for use in protocols.	
Touchscreen	The AAW $^{\text{TM}}$ Workstation has its own touchscreen interface to control it directly, in addition to using the Opentrons $^{\text{®}}$ App. Use the touchscreen to start protocol runs, check job status, and change settings right on the robot.	
Module caddies	The Opentrons® modules fit into caddies that occupy space below the deck. Caddies place the labware closer to the deck surface and allow for below-deck cable routing. Caddies enable even more module and labware configurations on the deck.	
Deck slot coordinates	Deck slots on the AAW $^{\text{\tiny TM}}$ Workstation are numbered with a coordinate system (A1–D4) which is similar to how wells are numbered on labware.	
Movable trash	The trash bin can go in multiple deck locations on the AAW^{TM} Workstation. The default location (slot A3) is the recommended position. The gripper can also be used to dispose of trash in the optional waste chute.	
Size and weight	The AAW™ Workstation measurement and weight is in the Safety Sheet. Installation tasks require the assistance of a lab partner.	

The $\mathsf{AAW}^{\scriptscriptstyle\mathsf{TM}}$ Workstation runs on our open-source software.

Safety Sheet

The AAW™ Workstation liquid handling robot has been designed for safe operation. Prior to use, read the Safety Sheet that has been shipped with workstations and modules. The Safety Sheet details the physical, environmental and electrical requirements and warnings in many languages. It should be stored near the AAW™ Workstation for easy access. Additional copies can be downloaded from the AAW™ Workstation - Assay Ready product page at SigmaAldrich.com. Using the device in a manner other than those specified in this user guide, may put the user and equipment at risk.

Installation and Relocation

Before taking delivery of the AAW™ Workstation, make sure that the lab or facility meets all the criteria in the listed below. Read this section completely before unboxing.

Location Requirements

Space is a valuable commodity in almost every lab. The AAW™ Workstation is designed to fit on half of a standard lab bench. Make sure there is a space that meets the criteria listed below.

Bench surface

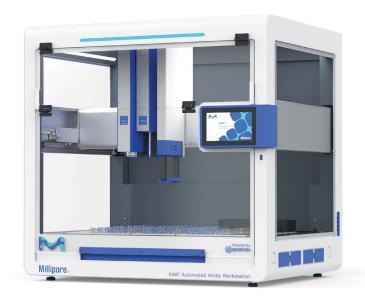
Stationary, sturdy, level, water-resistant surface. Tables or benches with wheels (even locking wheels) are not recommended. The AAW^{TM} Workstation moves quickly and has a lot of mass, which can shake or imbalance lightweight or movable tables.

Weight bearing

The workstation alone weighs over 86.18 kg (190 lb) and should only be lifted by two people working together. Place the workstation on a surface that can readily support its weight plus the weight of any modules, labware, liquids, or other lab equipment to be used in applications.

Operating space

The workstation base dimensions are shown in the <u>Safety Sheet</u>. An additional 20 cm (8 inches of side and back clearance is required for cables USB connections, and to dissipate exhaust from modules that heat and cool.



20 cm (8 inches)

-20 cm (8 inches)

-20 cm (8 inches)

FRONT

showing minimum side and back clearance.

(Right) Top view of AAW™ Workstation,

Power Consumption

The AAW™ Workstation should be connected directly into a wall outlet at or near the bench location. Only connect the AAW™ Workstation to circuits that can accommodate its maximum power draw. See the Safety Sheet for directions to electrical specifications.

Exact power consumption depends on:

- The amount and type of movement executed during a protocol
- The amount of time the robot spends idle
- The status of the lights on the robot
- How many instruments are attached

Remember to account for other electronics that consume power on the same circuit, including modules with their own power supplies. For example, the Thermocycler Module has a maximum power consumption (630 W) that is much greater than the workstation itself. Consult the facilities manager to make sure the desired location meets the equipment's power requirements.

Environmental Conditions

Environmental conditions for acceptable use and storage are in the Safety Sheet.

Recommended For System Operation

Ambient temperature	20 to 25 °C	
Relative humidity	40-60%, non-condensing	
Altitude	Approximately 500 m above sea level	

The performance of AAW™ Workstation has been validated in the conditions recommended for system operation, and operation in those conditions should provide optimal results. The AAW™ Workstation is safe to use in conditions acceptable for system operation, but results may vary. Do not power on or use the AAW™ Workstation in conditions outside of those bounds. The storage and transportation conditions only apply when the robot is completely disconnected from power and other equipment.

Unboxing

The User Kit, pipettes, gripper, and modules are shipped securely strapped to the top of the of the AAW™ Workstation main crate. Do not stack anything on top of the mini pallet. Do not unpack before the insaller arrives. The installer will unpack and set up the workstation and components initially.

Crate and Packing Material

We recommend keeping the crate's several large panels along with assorted shipping components and padding if storage space is available. The packaging is reusable, which can be used to ship the AAW^{TM} Workstation in the future (e.g., to a conference or a new facility). See Remove the Crate on page 11 for details on (dis)assembly of the crate.

User Kit Contents



(1) Emergency Stop Pendant



(1) USB Cable



(1) Power Cable



(1) Ethernet Cable



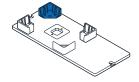
(4) Carrying Handles and Caps

(5) L-Keys Including

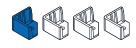
12 mm Hex, 1.5 mm Hex,

2.5 mm Hex, 3 mm Hex,

T10 Torx



(1) Deck Slot with Labware Clips



(4) Spare Labware Clips



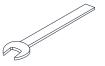
(1) Pipette Calibration Probe



(10) Spare Deck Slot Screws (M4x10 mm Socket Head)



(12) Spare Deck Clip Screws (M3x6 mm Socket Head)



(1) 19 mm Wrench



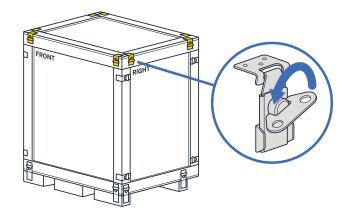
(1) 2.5 mm Hex Screwdriver

Remove the Crate

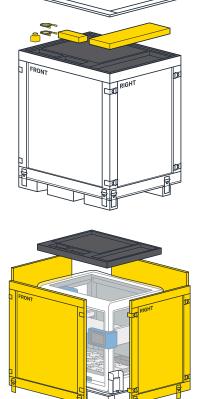
The AAW™ Workstation ships in a sturdy plywood crate. The shipping crate uses hook and latch clamps to secure the top, side, and bottom panels together. Using latches, instead of nails or screws to avoid using a crowbar (or a lot of force) to disassemble the crate, and can be reassemble later, if needed.

Note: Crate edges can get roughed up during shipping. We recommend using work gloves to protect hands from wood splinters.

1. Unlock the eight latches holding the top to the sides. To release the latches, flip the latch tab up and turn it to the left (counterclockwise). This action moves the clamp arm out of its corresponding retaining bracket. Flip the latch arm away from the crate.



- 2. Remove the top panel after releasing the latches.
- 3. Cut open the blue shipping bag, remove these items from the padding, and set them aside:
 - User Kit (tools, fasteners, etc.)
 - Power, Ethernet, and USB cables
 - Emergency Stop Pendant
- 4. Remove the top piece of foam padding.
- 5. Unlock the remaining 16 latches holding the side panels to each other and the base of the crate.
- 6. Remove the side panels and set them aside.

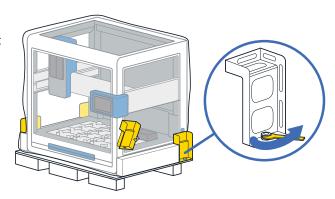


Release the AAW™ Workstation

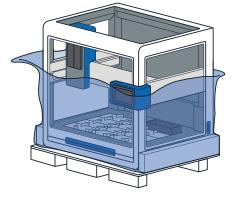
The workstation protective bag will be visible and attached to orange steel mounting components. The bag encloses the workstation and protects it from the outside environment. Steel brackets secure it to the bottom of the crate. Two shipping frames provide support, distributing its weight evenly, and keeping it rigid so it doesn't warp during shipping.

Continue to unpack the AAW™ workstation and get it off the crate base.

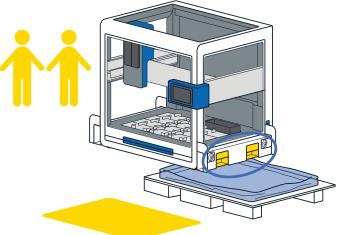
7. Using the 19 mm wrench from the User Kit, unbolt the brackets from the crate bottom. Discard the brackets, or save them for future use.



8. Pull or roll the shipping bag all the way down to expose the entire robot.



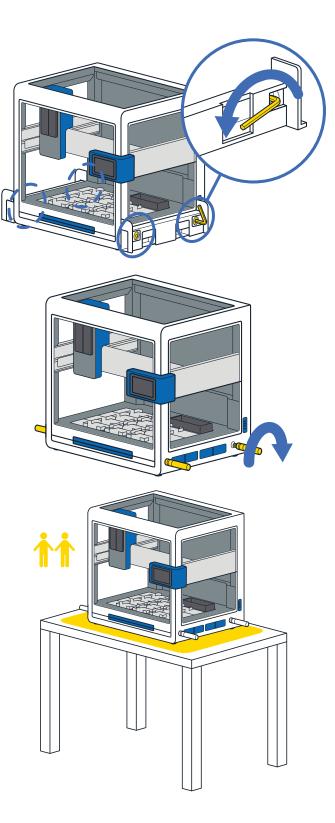
9. With help from a lab partner, grab the handholds in the orange shipping frames on either side of the robot's base, lift the AAW™ Workstation off the crate base, and set it down on the floor. Save or discard the crate base and shipping frame.



10. Using the 12 mm hex L-key from the User Kit, remove the four bolts holding the shipping frames to the AAW™ Workstation. Save or discard the frames and bolts.

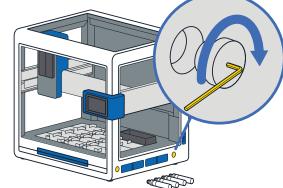
11. Locate the four aluminum handles in the User Kit. Screw the handles into the same locations that held the 12 mm shipping frame bolts.

12. With help from a lab partner, lift the AAW[™] Workstation by its carrying handles and move it to a workbench for final assembly.



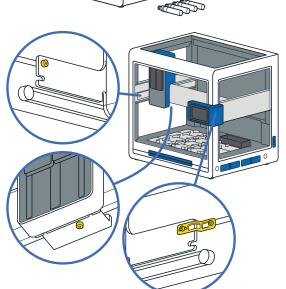
Final Assembly and Power

13. If the workstation is now in its final, working location, remove the carrying handles and replace them with the finishing caps. The caps close the handle openings in the frame and give a clean appearance. Return the handles to the User Kit for storage.



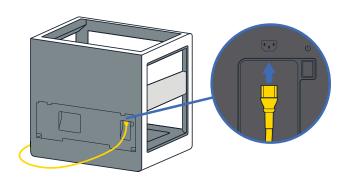
- 14. Using the 2.5 mm screwdriver from the User Kit, remove the locking screws from the gantry. These screws prevent the gantry from moving while in transit. The gantry locking screws are located:
 - On the left side rail near the front of the robot.
 - Underneath the vertical gantry arm.
 - On the right side rail near the front of the robot in an orange bracket. There are two screws here.

The gantry moves easily by hand after removing all the shipping screws.



- 15. Cut and remove the two rubber bands that hold the trash bin in place during shipping.
- 16. Firmly attach the power cord to the AAW™ Workstation and plug it into the wall outlet. Make sure the deck area is free of obstructions. Flip the power switch on the back left of the robot up, to the ON (|) position. Once powered on, the gantry moves to its home location and the touchscreen displays additional configuration instructions.

Now that the AAW $^{\text{TM}}$ Workstation is out of the box and ready to go, continue to First Run on page 15.



First Run

Perform basic setup on the touchscreen before connecting any other hardware to the AAW $^{\text{TM}}$ Workstation. The software will provide direction for connecting to the lab network, updating to the latest software, and giving the AAW $^{\text{TM}}$ Workstation a name.

Power On

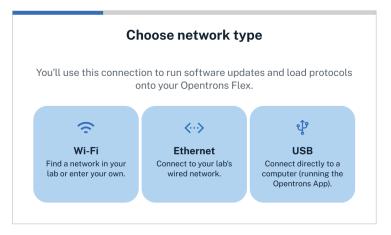
When the AAW $^{\text{TM}}$ Workstation is powered on, the AAW $^{\text{TM}}$ Workstation screen will appear.



Connect to a Network or Computer

Follow the prompts on the touchscreen to get the robot connected so it can check for software updates and receive protocol files. There are three connection methods: wireless network, Ethernet, and USB.

Network connection internet connectivity is required to set up the AAW^{TM} Workstation.



Wireless Network

Use the touchscreen to connect to wireless networks that are secured with WPA2 Personal authentication (most networks that only require a password to join fall under this category).

Note: Captive portals are not supported (networks that don't have a password but load a webpage to authenticate users after connecting).

Open Wireless networks are not recommended. Connecting to an open wireless network will allow anyone in range of the network signal to control the AAW™ Workstation without authentication.

To connect to a wireess network that uses enterprise authentication (including "eduroam" and similar academic networks that require a username and password), first connect to the Opentrons® App by Ethernet or USB to complete initial setup. Then connect to the enterprise wireless network in the **Networking Settings** for the AAW™ Workstation. To access the networking settings:

- 1. Click Devices in the left sidebar of the Opentrons® App.
- 2. Click the three-dot menu (:) and choose Robot Settings.
- 3. Click the Networking tab.

Select a network from the dropdown menu or choose "Join other network..." and enter its SSID. Choose the enterprise authentication method that the network uses. The supported methods are:

- EAP-TTLS with TLS
- EAP-TTLS with MS-CHAP v2
- EAP-TTLS with MD5
- EAP-PEAP with MS-CHAP v2
- EAP-TLS

Each of these methods requires a username and password, and depending on the exact network configuration may require certificate files or other options. Consult your facility's IT documentation or contact your IT manager for details of your network setup.

Ethernet

Connect the robot to a network switch or hub with an Ethernet cable. The Ethernet cable can be directly connected to the port on the computer, starting in robot system version 7.1.0.

USB

Connect the provided USB A-to-B cable to the workstation USB-B port, and to an open port on a computer. Use a USB B-to-C cable or a USB A-to-C adapter if the computer does not have a USB-A port.

To proceed with setup, the connected computer must have the Opentrons® App installed and running. For details on installing the Opentrons® App, see <u>The Opentrons® App on page 85</u>.

Install Software Updates

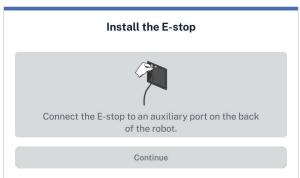
The AAW™ Workstation can check for software and firmware updates and download them if needed. If there is an update, it may take a few minutes to install. Once the update is complete, the robot will restart.

Attach Emergency Stop Pendant

Connect the included Emergency Stop Pendant (E-stop) to an auxiliary port (AUX-1 or AUX-2) on the back of the workstation.

Before connecting the Emergency Stop Pendant.

After connecting the Emergency Stop Pendant.





Attaching and enabling the E-stop is mandatory. For more information on using the E-stop during robot operation, see the <u>Emergency Stop Pendant on page 31</u>.

Assign a Name

Naming the AAW™ Workstation allows easily identification it in the lab environment if there are multiple AAW™ Workstations on the network. Assign unique names. The AAW™ Workstation Dashboard will appear on the touchscreen.

Instrument Installation and Calibration

After initial robot setup, the next step is to attach instruments to the robot and calibrate them.

To install an instrument, first tap on Instruments on the touchscreen or go to the Pipettes and Modules section of the device detail screen in the Opentrons® App. Choose an empty mount and select either Attach Pipette or Attach Gripper. If the mount already occupied, detach the pipette or gripper first.

Note: The overall installation process is the same regardless of whether the touchscreen or the Opentrons® App is used. Whatever device choose will control the installation process until it is complete or canceled.

Beginning on the touchscreen, the app will show the robot as being "busy". If beginning in the app, the touchscreen will show a modal indicating that instrument installation is in progress.

The exact installation process varies depending on the instrument being attached, as covered in the sections that follow. All instruments have an automated calibration procedure, which should be performed immediately after installation.

Pipette Installation

To install a pipette, follow steps on the touchscreen or in the Opentrons® App.

1. Choose pipette type.

Choose between 1- or 8-Channel Pipette and 96-Channel Pipette. Attaching the 96-Channel Pipette requires a few additional steps because it attaches to a special mounting plate that spans both pipette mounts.

2. Prepare for installation.

Remove labware from the deck and clean up the working area to make attachment and calibration easier. Also gather the needed equipment, such as the calibration probe, hex screwdriver, and mounting plate (for the 96-Channel Pipette).

3. Connect and secure the pipette.

The gantry will move to the front of the robot so the pipette can be attached.

The 1- and 8-Channel Pipettes connect directly to a pipette mount. The 96-Channel Pipette requires a mounting plate. In order to attach the mounting plate, first disconnect the z-axis carriage for the right pipette mount.

Connect the pipette to the chosen pipette mount and secure its screws.

Note: The 1- and 8-Channel Pipette screws need moderate inward pressure to engage. Do not overtighten!

4. Run automated calibration.

To calibrate the pipette, attach the calibration probe to the indicated pipette nozzle. The pipette will automatically move to touch certain points on the deck and save these calibration values for future use. Once calibration is complete and the probe has been removed, the pipette will be ready for use in protocols.

AAW™ Flex Gripper Installation

Installation can be performed from the touchscreen or in the Opentrons® App.

1. Prepare for installation.

Remove labware from the deck and clean up the working area to make attachment and calibration easier. Also gather the required hex screwdriver and make sure that the calibration pin is in its storage area on the gripper.

2. Connect and secure the gripper.

The gantry will move to the front of the robot so the gripper can be attached. Connect the gripper to the extension mount and secure its screws.

Run automated calibration.

To calibrate the AAW $^{\text{TM}}$ Flex Gripper, insert the calibration pin in the front jaw. The gripper will automatically move to touch certain points on the deck and save these calibration values for future use. Then repeat the same process with the calibration pin in the back jaw. Once calibration is complete and the pin is back in its storage location, the gripper will be ready for use in protocols.

Relocating the AAW™ Workstation

Short Moves

A short move spans a range of distances from "let's just move it over a little bit" to across the lab, down the hall, or another floor in the same building. In these cases, move the AAW™ Workstation by hand. Transporting it on a hand cart is also a good option.

Warning: The AAW[™] Workstation weighs approximately 88.5 kg and requires two people to lift and move it safely.

Reattach the lift handles (see illustration <u>page 13</u>) to move the AAW™ Workstation to a new, nearby location. Lifting and carrying by the handles is the right way to move the robot short distances. Remove the handles and store them in the User Kit after the move is complete. To prevent damaging the robot, always use the lift handles to pick it up and move it.

Warning: Never grab the frame to lift or move the robot.

Long-Distance Moves

A long-distance move is transporting the AAW™ Workstation off the grounds of your facility. In this case, pack the AAW™ Workstation to protect it from the elements, shocks, and rough movements that may occur while in transit.

If the shipping crate and internal supports were stored, it can be repackage it in these materials for a long-distance move. Follow <u>Unboxing on page 9</u> in reverse order to prepare the AAWTM Workstation for a long-distance move.

- Disconnect the power and network cable, if attached.
- · Remove all attached hardware and labware.
- Reattach the deck plates.
- Lock the gantry (see General Moving Advice on page 19).

If the original crate is available:

- Reattach the shipping frame to the AAW[™] Workstation and secure it to the pallet base using the L-brackets.
- Add padding and reassemble the shipping crate.

If the original crate and related material is not available, contact a reputable shipping company. They can manage the packing, transportation, and delivery process.

General Moving Advice

The AAW™ Workstation is a sturdy and well-built machine, but it is also a precise scientific instrument designed to exacting tolerances. As a result, treat it with care when relocating it. Follow the guidance provided here and using common sense about how to transport an expensive piece of laboratory equipment.

Please direct questions or concerns about relocating to SigmaAldrich.com/TechService.

Required Steps Before Moving

Disconnect Power and Network Cables

- Turn off the power and unplug it from the power supply.
- Disconnect the Ethernet or USB cable, if used.

Lock the Gantry

Reinsert the locking screws to hold the gantry in place. The gantry locking points are located:

- On the left side rail near the front of the robot.
- Underneath the vertical gantry arm.
- On the right side rail near the front of the robot. Locking this part of the gantry requires the small orange bracket and two locking screws.

Home the Gantry

It may not be necessary to lock the gantry if moving the robot to a nearby location. If the gantry is not locked, use the touchscreen or the Opentrons® App to send the gantry to its "home" position before powering it down.

To home the gantry via the touchscreen, tap the three-dot menu (i) and then tap **Home Gantry**.

To home the gantry via the Opentrons® App:

- 1. Click Devices.
- 2. Click on the AAW™ Workstation in the device list.
- 3. Click the three-dot menu (:) and then click **Home Gantry**.

Remove Modules

In-deck modules and other attachments add extra weight to the AAW™ Workstation. They also affect the robot's center of gravity, which can make it feel "tippy" when lifting it. To help lighten and balance the robot, remove any attached instruments and labware before picking it up.

Reinstall Deck Slots

We recommend reattaching the deck slots for a long-distance move. Securing the slots in their original locations helps prevent accidental loss.

Reattaching the deck slots for short moves around the lab is optional.

Post-Move Recalibration

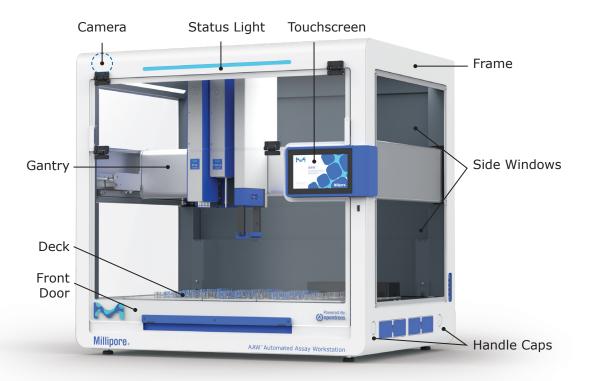
Recalibrate any instruments and modules after reinstalling them. For more details on module calibration, see <u>Modules on page 35</u>.

System Description

The deck, gantry, and instrument mounts enable the use of precision liquid- and labware-handling components. The on-device touchscreen enables running protocols and checking on the robot's status without needing to bring a computer to the lab bench. Wired and wireless connectivity enables additional control from the Opentrons® App (see The Opentrons® App On page 85) and extending the system's features by attaching peripherals (see Modules on page 35).

Physical Components

Locations of the physical components of AAW™ Workstation.



Frame and Enclosure

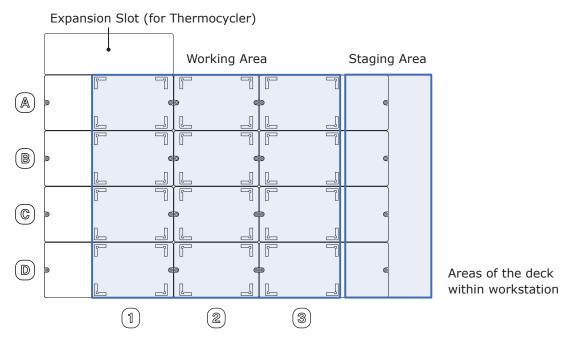
The frame of the AAW™ Workstation provides rigidity and structural support for its deck and gantry. All of the mechanical subsystems are situated on and mounted to the main frame. The frame is constructed primarily of sheet metal and aluminum extrusions.

The metal frame has openings for side windows and a front door made of transparent polycarbonate to view inside the AAW™ Workstation. The front door hinges open for access to the interior of the system. With the front door open, instruments, modules, and deck fixtures can be attached; prepare the deck before a protocol; or manipulate the state of the deck during a protocol.

White LED strips on the inside top edges of the frame provide software-controllable ambient lighting. A 2-megapixel camera can photograph the deck and working area for recording and tracking protocol execution.

Deck and Working Area

The deck is the machined aluminum surface on which automated science protocols are executed. The deck has 12 main ANSI/SLAS-format slots that can be reconfigured to hold labware, modules, and consumables. The deck slots are identified by a coordinate system, with slot A1 at the back left and slot D3 at the front right.



The working area is the physical space above the deck that is accessible for pipetting. Labware placed in slots A1 through D3 are in the working area.

The AAW™ Workstation comes with removable deck slots for all 12 positions in the working area. Each deck slot has corner labware clips for securely placing labware on the deck.

Reconfigure the deck by replacing slots with other deck fixtures, including the movable trash, waste chute, and module caddies. The expansion slot behind A1 is only used to make additional room for the Thermocycler Module, which occupies slots A1 and B1.

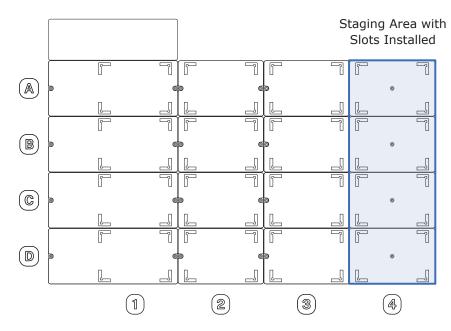
Note: Deck slots are interchangeable within a column (1, 2, or 3) but not across columns; column 1 and column 3 slots are distinct pieces despite their similar size. Orient the blue labware clip to the back left.

The deck slots should be installed in locations for standalone labware. The deck and items placed on it remain static, unless moved by the gripper or manual intervention.

Staging Area

The staging area is additional space along the right side of the deck. Store labware in this location after installing staging area slots. Labware placed in slots A4 through D4 are in the staging area. The pipettes cannot reach into the staging area, but the gripper can pick up and move labware to and from this location. Adding extra slots helps keep the working area available for the equipment used in automated protocols.

Staging area slots are included in certain workstation configurations and are also available for purchase (see Product Ordering on page 103).



Deck Fixtures

Fixtures are hardware items that replace standard deck slots. They customize the deck layout and add functionality to the AAW™ Workstation. Currently, deck fixtures include the staging area slots, the internal trash bin, and the external waste chute. Fixtures can only be installed in a few specific deck slots. The following table lists the deck locations for each fixture.

Fixture	Slots
Staging area slots	A3-D3
Trash bin	A1-D1 and A3-D3
Waste chute	D3 only
Waste chute with staging area slot	D3 only

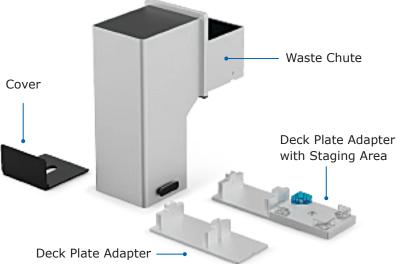
Fixtures are unpowered. They do not contain electronic or mechanical components that communicate their current state and deck location to the robot. Use the deck configuration feature to communicate what fixtures are attached to the deck and where they're located.

Access the deck configuration settings from the touchscreen via the three-dot (;) menu and from the Opentrons® App. See <u>Deck Configuration on page 82</u>, to configure the deck from the touchscreen.

Waste Chute

The waste chute transfers liquids, tips, tip racks, and well plates from the enclosure to a trash receptacle placed below its external opening. The waste chute attaches to a deck plate adapter that fits in slot D3. It also comes with a special window half panel that lets the chute extend out of the front.

Waste Chute Components



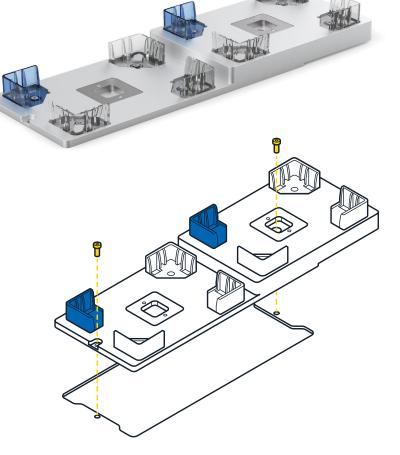
Staging Area Slots

Staging area slots are ANSI/SLAS compatible deck pieces that replace standard slots in column 3 and add new slots to the staging area without losing space in the working area. Install a single slot or a maximum of four slots to create a new column (A4 to D4) along the right side of the deck. However, replacing deck slot A3 requires moving the trash bin.

By adding staging area slots to the deck, the robot can store more labware and operate more efficiently.

Slot Installation

To install, remove the screws that attach a standard slot to the deck and replace it with the staging area slot. After installation, use the touchscreen or Opentrons® App to specify the added staging area slot to the deck.



Slot Compatibility

Staging area slots are compatible with the instruments, modules, and labware listed below.

Component	Staging Area Compatibility		
AAW™ Flex Gripper	The AAW™ Flex Gripper can move labware to or from staging area slots.		
AAW™ Pipettes	ttes The pipettes cannot reach the staging area. Use the gripper to move tip racks and labware from the staging area to the working area before pipetting.		
Modules	The Magnetic Block GEN1 can be placed in column 3 on top of a staging area slot. Modules are not supported in column 4.		
	Powered modules such as the Heater-Shaker and Temperature Module fit into caddies that can be placed in column 3. A staging area slot cannot be added to a position occupied by a module caddy.		
Labware	Staging area slots have the same ANSI/SLAS dimensions as standard deck slots. Use gripper-compatible labware in the staging area, or manually add and remove labware from this location.		

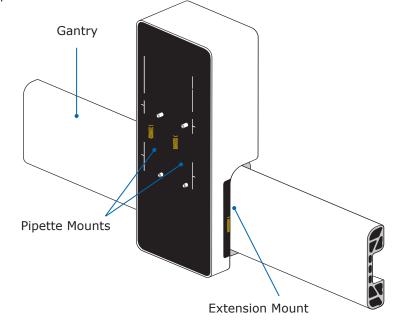
Gantry

Attached to the frame is the gantry, which is the robot's movement and positioning system.

The gantry moves separately along the x- and y-axis to position the pipettes and gripper at precise locations for protocol execution. Movement along these axes is precise to the nearest 0.1 mm. The gantry is controlled by 36 VDC hybrid bipolar stepper motors.

The pipette mounts and the extension mount are attached to the gantry. These move along the z-axis to position the pipettes and gripper at precise locations for protocol execution. Movement along this axis is controlled by 36 VDC hybrid bipolar stepper motors.

The electronics contained in the gantry provide 36 VDC power and communications to the pipettes and gripper, when attached.



Touchscreen and LED Displays

The primary user interface is the 7-inch LCD touchscreen, located on the front right of the workstation. The touchscreen glass has been treated for scratch and damage resistance. Features available through the touchscreen include:

- Protocol management
- Protocol setup, execution, and monitoring
- Labware management
- Robot settings
- System software and firmware updates
- Operation logs and error notifications

For more information on using the touchscreen, see <u>Touchscreen Operation on page 72</u>.

The status light is a strip of LEDs along the top front of the robot that provides at-a-glance information about the robot. Different colors and patterns of illumination can communicate various success, failure, or idle states:

LEI	D Color	LED Pattern	Robot Status
0	White Neutral states	Solid	Powered on and not running a protocol
		Pulsing	Robot is busy (e.g., updating software or firmware, setting up protocol run, canceling protocol run)
•	Green Normal states	Blinks twice	Action is complete (e.g., protocol stored, software updated, instrument attached or detached)
		Solid	Protocol is running
		Pulsing	Protocol is complete
•	Blue Mandatory states	Pulsing	Protocol is paused
•	Yellow Abnormal states	Solid	Software error
•	Red Emergency states	Blinks three times, repeatedly	Physical error (e.g., instrument crash)

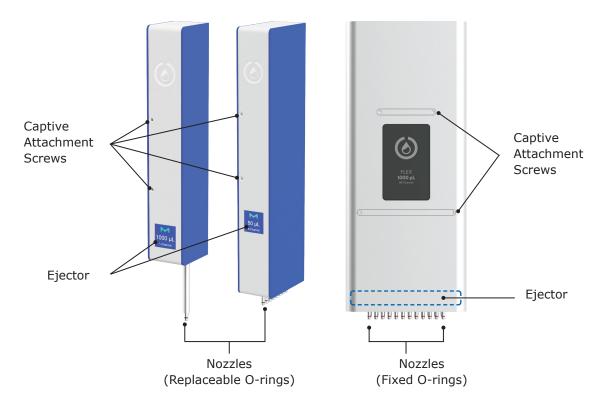
The status light can also be disabled in settings.

Pipettes

Pipettes are configurable devices used to move liquids throughout the working area during the execution of protocols. There are several AAW $^{\text{TM}}$ Workstation pipettes, which can handle volumes from 1 μ L to 1000 μ L in 1-, 8-, or 96-channels:

- AAW[™] 1-Channel Pipette (1–50 μL)
- AAW™ 1-Channel Pipette (5-1000 μL)
- AAW[™] 8-Channel Pipette (1–50 μL)
- AAW[™] 8-Channel Pipette (5–1000 µL)
- 96-Channel Pipette (5-1000 μL)

Pipettes attach to the gantry using captive screws on the front of the pipette. 1-channel and 8-channel pipettes each occupy one pipette mount (left or right); the 96-channel pipette occupies both mounts. For details on installing pipettes, see <u>Instrument Installation and Calibration on page 17</u>.



The pipettes pick up disposable plastic tips by pressing them onto the pipette nozzles, and then use the tips to aspirate and dispense liquids. The amount of total force required for pickup increases as more tips get picked up simultaneously. For smaller numbers of tips, the pipette attaches tips by pushing each pipette nozzle down into a tip. To achieve the necessary force to pick up a full rack of tips, the 96-channel pipette also pulls the tips upward onto the nozzles. This pulling action requires placing tip racks into a tip rack adapter, rather than directly in a deck slot. To discard tips (or return them to their rack), the pipette ejector mechanism pushes the tips off of the nozzles.

Pipette Specifications

The AAW™ Workstation pipettes are designed to handle a wide range of volumes. Because of their wide overall range, they can use multiple sizes of tips, which affect their liquid-handling characteristics. The AAW™ pipettes have been tested for accuracy and precision in a number of tip and liquid volume combinations:

Pipette	Tip Capacity	Tested Volume	Accuracy %D	Precision %CV
AAW™	50 μL	1 μL	8.00%	7.00%
1-Channel	50 μL	10 μL	1.50%	0.50%
50 μL	50 μL	50 μL	1.25%	0.40%
	50 μL	5 μL	5.00%	2.50%
AAW™ 1-Channel	50 μL	50 μL	0.50%	0.30%
1000 µL	200 μL	200 μL	0.50%	0.15%
	1000 μL	1000 μL	0.50%	0.15%
AAW^{TM}	50 μL	1 μL	10.00%	8.00%
8-Channel	50 μL	10 μL	2.50%	1.00%
50 μL	50 μL	50 μL	1.25%	0.60%
	50 μL	5 μL	8.00%	4.00%
AAW™ 8-Channel	50 μL	50 μL	2.50%	0.60%
1000 µL	200 μL	200 μL	1.00%	0.25%
	1000 μL	1000 μL	0.70%	0.15%
	50 μL	5 μL	10.00%	5.00%
Opentrons® 96-Channel	50 μL	50 μL	2.50%	1.25%
1000 µL	200 μL	200 μL	1.50%	1.25%
	1000 μL	1000 μL	1.50%	1.50%

Keep this accuracy information in mind when choosing pipette tips. Use the smallest tips that meet the needs of the protocol.

Note: Volumetric testing of pipettes has been done to ensure that they meet the accuracy and precision specifications listed above. It is not necessary to calibrate the volume that the pipettes dispense before use. Positional calibration is required.

The Opentrons® Care Plus services include yearly pipette replacement and certificates of calibration. See Maintenance and Service on page 90.

Pipette Calibration

The User Kit includes a metal pipette calibration probe for positional calibration. During protocol runs, safely store the probe on the magnetic holder on the front pillar of the robot. During the calibration process, attach the probe to the appropriate nozzle and lock it in place. The robot moves the probe to calibration points on the deck to measure the pipette's exact position.

Pipette Tip Rack Adapter

The Opentrons® 96-Channel Pipette ships with four tip rack adapters. These are precision formed aluminum brackets that can be placed on the deck. The adapters hold 50 μ L, 200 μ L, and 1000 μ L tip racks.

Because of the force involved, only the 96-channel pipette requires an adapter to attach a full tip rack properly. During the attachment procedure, the pipette moves over the adapter, lowers itself onto the mounting pins, and pulls tips onto the pipettes by lifting the adapter and tip rack. Pulling the tips, rather than pushing, provides the leverage needed to secure tips to the pipettes and prevents warping the deck surface. When finished, the 96-channel pipette lowers the adapter and empty tip rack onto the deck. See Pipette Tips and Tip Racks on page 49.



Partial Tip Pickup

The 96-channel pipette can pick up a full rack of tips or a smaller number of tips. This increases the number of applications that can be performed with the 96-channel pipette, since it occupies both pipette mounts.

Currently, the 96-channel pipette supports partial tip pickup for 8 tips in a column layout. In this configuration, the pipette either uses its leftmost nozzles to pick up tips right-to-left from a tip rack, or its rightmost nozzles to pick up tips left-to-right from a tip rack.

When picking up fewer than 96 tips from a tip rack, the rack must be placed directly on the deck, not in the tip rack adapter.

Pipette Sensors

AAW™ Workstation pipettes have a number of sensors that detect and record data about the status of the pipette and any tips it has picked up.

Capacitance Sensors

In combination with a metal probe or conductive tip, the capacitance sensors detect when the pipette makes contact with something. Detection of contact between the metal probe and the deck is used in the automated pipette calibration and module calibration processes.

The 1-channel pipettes have one capacitance sensor. Multi-channel pipettes have two; on channels 1 and 8 of 8-channel pipettes, and on channels 1 and 96 (positions A1 and H12) of the 96-channel pipette.

Optical Tip Presence Sensors

A photointerruptor switch detects the position of the pipette tip ejector mechanism, confirming whether tips were successfully picked up or dropped. The 1-channel, 8-channel, and 96-channel pipettes all have a single optical sensor that monitors tip attachment across all channels.

Presence Sensors

Flex pipettes use internal pressure sensors to detect liquid in well plates, reservoirs, and tubes. Liquid detection takes place as a pipette approaches the surface of a liquid. Sensors in the pipettes detect pressure changes relative to ambient pressure. A particular change in pressure tells the robot that liquid is present in a well and the pipette tip is in contact with the liquid's surface.

The 1-channel pipettes have one pressure sensor. The 8-channel pipette pressure sensors are on channels 1 and 8 (positions A1 and H1). The 96-channel pipette pressure sensors are on channels 1 and 96 (positions A1 and H12). Other channels on multi-channel pipettes do not have sensors and cannot detect liquid.

Pipette Firmware Updates

AAW™ Workstation automatically updates pipette firmware to keep it in sync with the robot software version. Pipette firmware updates are typically quick, and occur whenever a pipette is attached or the robot restarts.

If the pipette firmware and robot software versions get out of sync, manually update the firmware in the Opentrons® App.

- 1. Click Devices.
- 2. Click on the AAW™ Workstation in the device list.
- 3. Under **Instruments and Modules**, the out-of-sync pipette will show a warning banner reading "Firmware update available." Click **Update Now** to begin the update.

To view the currently installed firmware version of any attached pipette from the touchscreen, go to **Instruments** and tap the pipette name. In the Opentrons® App, find the pipette card under **Instruments and Modules**, click the three-dot menu (i), and then click **About Pipette**.

AAW™ Flex Gripper

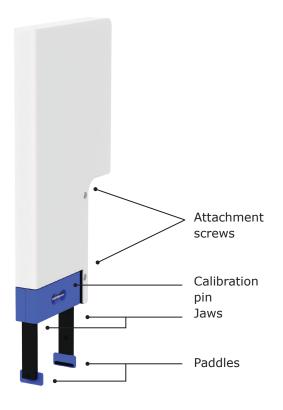
The gripper moves labware throughout the working area and staging area during the execution of protocols. It is attached to the extension mount, which is separate from the pipette mounts. The gripper can be used with any pipette configuration. For details on installing the gripper, see <u>AAW™ Flex Gripper Installation on page 18</u>.

The AAW™ Flex Gripper can move labware across the deck and onto or off of modules. It can also manipulate certain fully skirted well plates, deep well plates, and tip racks. For more details on what labware the gripper can move, see <u>Labware and the AAW™ Flex Gripper on page 55</u>. The Millipore® Protocol Library includes a labware library section with materials used to run protocols. Additionally, the broader Opentrons® Labware Library is also available.

Specifications

The jaws perform the primary motion of the gripper, which is to open or close two parallel paddles to apply or release force on the sides of labware. Movement of the jaws is controlled by a 36 VDC brushed motor connected to a rack-and-pinion gear system.

To move a piece of labware that has been gripped by the jaws, the gantry lifts the gripper along the z-axis, moves it laterally, and then lowers it into the labware's new position.



Calibration

The AAW™ Flex Gripper includes a metal calibration pin. The calibration pin is located in a recessed storage area on the lower part of the gripper. A magnet holds the pin in place. To remove the calibration pin, grasp it with your fingers and pull gently. To replace the pin, put it back in the storage slot. It is secure when it snaps into place.

When calibrating the AAW™ Flex Gripper, attach the pin to each jaw in turn. The robot moves the pin to calibration points on the deck to measure the gripper's exact position.

During protocol runs, place the pin in its storage area for safekeeping. To get a replacement pin, contact us at SigmaAldrich.com/TechService.

Firmware Updates

The AAW™ Workstation automatically updates the gripper firmware to keep it in sync with the robot software version. Gripper firmware updates are typically quick, and occur whenever the gripper is attached or the robot restarts.

If the AAW™ Flex Gripper firmware and robot software versions get out of sync, manually update the firmware in the Opentrons® App.

- 1. Click **Devices**.
- Click on the AAW™ Workstation in the device list.
- 3. Under **Instruments and Modules**, the out-of-sync gripper will show a warning banner reading "Firmware update available." Click **Update Now** to begin the update.

The currently installed firmware version of the AAW™ Flex Gripper can be viewed on the touchscreen. Go to **Instruments** and tap the gripper. From the Opentrons® App, find the gripper card under **Instruments and Modules**, click the three-dot menu (i), and then click **About Gripper**.

Emergency Stop Pendant

The Emergency Stop Pendant (E-stop) is a dedicated hardware button for quickly stopping robot motion. A functional, disengaged E-stop is required to be attached at all times. When the stop button is pressed, the AAW™ Workstation cancels any running protocol or setup workflow as quickly as possible and prevents most robot motion. Pressing the E-stop does not stop power to the workstation.

When to use the E-stop

- When there is imminent risk of injury or harm to a user.
- When there is imminent risk of damage to the robot or other hardware.
- When samples or reagents are in imminent danger of contamination.
- · After a hardware collision.

Ideally it should not be necessary to press the E-stop (except during infrequent hardware quality testing).

Do not use the E-stop to cancel normal, expected operations. Instead, use the software button on the touchscreen or in the Opentrons® App. Pausing via software will resume or cancel the protocol, whereas pressing the E-stop always cancels the protocol immediately.

Engaging and Releasing the E-stop

The E-stop has a press-to-engage, twist-to-release mechanism.

Engage: Push down firmly on the red button. The AAW™ Workstation will enter the stopped state.

Resolve: Once stopped, safely address any problems in the working area, such as clearing spills, removing labware, or moving the gantry (it should move freely and easily by hand).

Release: Twist the button clockwise. It will pop up to its disengaged position.

Reset: On the touchscreen or in the Opentrons® App, confirm that a are ready for AAW™ Workstation to resume motion. The gantry will return to its home position and module activity will resume.

In the stopped state, AAW™ Workstation and connected hardware will behave as follows:

Hardware	Behavior		
Gantry	Automated horizontal motion is halted.Manual horizontal motion is allowed.		
Pipettes	 Vertical motion of pipettes is halted. The motor brakes on vertical axes are engaged to prevent pipettes from falling. Plunger motion and tip pickup is halted. 		
AAW™ Flex Gripper	 Vertical motion of the gripper is halted. The motor brake on the vertical axis is engaged to prevent the gripper from falling. The jaw motors that exert gripping force remain enabled, so the gripper will not drop labware it may be carrying. 		
Heater-Shaker Module	 The shaker stops and homes. The labware latch opens. Heating is disabled.		
Temperature Module	Heating or cooling is disabled.		
Thermocycler Module	Heating or cooling is disabled.		
Status light	The light turns red.		
Touchscreen	 A cancellation message takes over the screen. An on-screen indicator shows when the stop button has been disengaged. 		

Connections

Power Connection

Before attempting to plug the AAW™ Workstation in, please read the Safety Sheet for electrical requirements and other warnings. The Safety Sheet was provided with the AAW™ Workstation, and can be downloaded from the product page at SigmaAldrich.com. Store the Safety Sheet near the workstation.

There is also a battery to power the robot's real-time clock when not connected to mains power. The battery is located inside the touchscreen enclosure. Contact SigmaAldrich.com/TechService before attempting to change the battery.



Ports AUX-1, AUX-2, USB-B, Ethernet

USB and Auxiliary Connections

The AAW™ Workstation has 10 USB ports located in different areas of the workstation.

The 8 rear USB-A ports (numbered USB-1 through USB-8) and 2 auxiliary ports (M12 connectors numbered AUX-1 and AUX-2) are for connecting AAW[™] modules and accessories. See Modules on page 104 for more information on connecting these devices and using them in the protocols.

The rear USB-B port is for connecting the robot to a laptop or desktop computer, to establish communication with the Opentrons® App running on the connected computer. The front USB-A port (USB-9), located below the touchscreen display, has the same functionality as the rear USB-A ports.

Note: The USB ports are power-limited to protect the robot and connected devices. Power delivery is split internally into three port groups: the left rear USB-A ports (USB-1 through USB-4), the right rear USB-A ports (USB-5 through USB-8), and the front USB-A port. Each of these groups will deliver a maximum of 500 mA to connected USB 2.0 compatible devices.

Network Connections

The AAW™ Workstation can connect to a local area network through a wired (Ethernet) or wireless connection. The Ethernet port is located on the rear of the workstation. Connect it to an Ethernet hub or connect it directly to an Ethernet port on the computer.

The internal wireless network module supports 802.11 ac/a/b/g/n networks with a dual-band 2.4/5 GHz antenna.

System Specifications

The <u>Safety Sheet</u> contains specifications for electrical, dimensional and environmental conditions.

General Specifications

Deck slots	12 ANSI/SLAS-compatible slots in working area (accessible to pipettes)	
	4 additional slots for staging tips and labware (accessible only to gripper)	
Touchscreen	7-inch LCD touchscreen with scratch-resistant and damage-resistant glass	
Wireless network	802.11 ac/a/b/g/n dual-band (2.4/5 GHz)	
Ethernet	100 Mbps	
USB	9 USB-A ports	
	1 USB-B port	
	USB 2.0 speed	
Camera	2 MP, photo and video	
Mains supply voltage fluctuation	±10%	
Mains supply frequency fluctuation	±5%	
Distribution system	TN-S	
Short-circuit supply current	6.3 A	
Frame composition	Rigid steel and CNC aluminum design	
Window composition	Removable polycarbonate side windows and front door	
Ventilation requirements	Minimum of 20 cm (8 inches) is required between the workstation and the wall	
Connected PC requirements	The Opentrons® App runs on:	
	Windows 10 or later	
	MacOS 10.10 or later	
	• Ubuntu 12.04 or later	

Certifications

Certifications complete $\,$ CE, ETL, FCC, ISO 9001 $\,$

Not certified/validated IVD, GMP

Certification information is printed on a sticker on the back of AAWTM Workstation, near the on/off switch. Also, see <u>Safety Sheet on page 7</u>.

Serial Number

The AAW™ Workstation serial number provides the robot's date of production and other information. For example, the serial number FLXMA1020251007001 would indicate:

Characters	Category	Meaning
FLXM	Model	The robot is an AAW™ Workstation.
A10	Version	A code for the production version of the workstation.
2025	Year	The robot was made in 2025.
10	Month	The robot was made in October.
07	Day	The robot was made on the 7th day of the month.
001	Unit	A unique number for robots made on a certain day.

To find the serial number for the AAW^{TM} Workstation:

- On the certification sticker on the back of the AAW™ Workstation, near the on/off switch.
- On the reverse side of the touchscreen (towards the working area).
- In the Opentrons® App under **Devices** > **AAW™ Workstation** > **Robot Settings** > **Advanced**.

Modules

The AAW™ Workstation can be combined with a number of hardware modules. All modules are peripherals that occupy deck slots, and most are controlled by the robot over a USB connection.

For details on integrating modules into protocols, see <u>Protocol Designer on page 64</u> or the online <u>Python Protocol API documentation</u>.

Supported Modules

The AAW™ Workstation is compatible with four types of modules:

- The Opentrons® Heater-Shaker Module provides on-deck heating and orbital shaking. The module can be heated to 95 °C, and can shake samples from 200-3000 rpm.
- The Opentrons® Magnetic Block is a passive device that holds labware close to its high-strength neodymium magnets.
- The Opentrons® Temperature Module is a hot and cold plate module that is able to maintain steady state temperatures between 4-95 °C.
- The Opentrons® Thermocycler Module provides on-deck, fully automated thermocycling, enabling automation of upstream and downstream workflow steps and is fully compatible with the AAW™ Flex Gripper.
- The HEPA (High Efficiency Particulate Air) module is a bolt-on addition to the top of the enclosure. Along with the built-in circulating fan, this addition facilitates contamination-free cell culture on the robotic deck.

Module Caddy System

Compatible modules are designed to fit into caddies that occupy space below the deck. This system allows labware on top of modules to remain closer to the deck surface, and it also allows for below-deck cable routing so the deck stays tidy during protocol runs.







Heater-Shaker Module Caddy

Temperature Module Caddy

Thermocycler Module Caddy

To fit a module into the deck surface, place it into the corresponding module caddy. Each type of compatible module has its own caddy design that aligns the module and labware precisely with the surrounding deck. Caddies for modules that occupy a single slot can be placed anywhere in column 1 or 3; the Thermocycler can only be placed in slots A1 and B1 simultaneously.

Note: The Magnetic Block sits directly on the deck surface instead of using a caddy because it does not require power or USB cable routing.

To install a module caddy:

- 1. Remove any deck slots from the location where the module will go.
- 2. Seat the module into its caddy and tighten its anchors.
- 3. Route the module power and USB cables through the side covers, up through the empty deck slot, and attach them to the module.
- 4. Seat the module caddy into the slot and screw it into place.

For installation instructions, consult the Quick Start Guide shipped with the module or locate the appropriate module section in this chapter.

Module Calibration

When first installing a module on the AAWTM Workstation, run automated positional calibration. This process is similar to positional calibration for instruments, and ensures that AAWTM robot moves to the exact correct locations for optimal protocol performance. During calibration, the robot will move to locations on a module calibration adapter, which looks similar to the calibration squares that are part of removable deck slots. The modules below require calibration. The Magnetic Block is ready for use as can be placed on the deck.



Heater-Shaker
Calibration Adapter



Temperature Calibration Adapter



Thermocycler Calibration Adapter

When to Calibrate Modules

The module requires calibration before it can be used. The AAW™ Workstation will automatically prompt to perform calibration when it detects a module is connected and powered on, but doesn't have any stored calibration data.

Once the calibration is completed, the data and module serial number are stored for future use. Recalibration is not needed unless the calibration data is deleted in settings. Modules can be powered on and off, or even move it to another deck slot, without needing to recalibrate. Recalibration can be done any time from the module card in the Opentrons® App. Recalibration is not available from the touchscreen.

How to Calibrate Modules

Instructions on the calibration procedure is available on the touchscreen or in the Opentrons® App.

- 1. Gather the required equipment, including the module calibration adapter and pipette calibration probe.
- Place the calibration adapter on the module surface and ensure that it is completely level.Some modules may require the adapter to be fastened to the module. Attach the calibration probe to a pipette.
- 3. The AAW™ Workstation will automatically move to touch certain points on the calibration adapter and save these calibration values for future use.

Once calibration is complete remove the adapter and probe. The module will be ready for use in protocols.

The module calibration data can be viewed and managed in the Opentrons® App. Go to **Robot Settings** > **Calibration** tab.

Opentrons® Heater-Shaker GEN1 Module

Features

The Opentrons Flex[™] Heater-Shaker GEN1 Module provides on-deck heating and orbital shaking. The module can be heated to 95 °C, with the following temperature profile:

• Temperature range: 37-95 °C

• Temperature accuracy: ±0.5 °C at 55 °C

 \bullet Temperature uniformity: ± 0.5 °C at 55 °C

• Ramp rate: 10 °C/min

The module can shake samples from 200-3000 rpm, with the following shaking profile:

Orbital diameter: 2.0 mm
Orbital direction: Clockwise
Speed range: 200-3000 rpm
Speed accuracy: ±25 rpm



The module has a powered labware latch for securing plates to the module prior to shaking.

Thermal Adapters

A compatible thermal adapter is required for adding labware to the Heater-Shaker. All four (4) thermal adapters are included with AAW™ Workstation - Assay Ready. Adapters can be purchased directly from <u>SigmaAldrich.com</u>. See <u>Product Ordering</u>. Thermal Adapters include:



Universal Flat Adapter



PCR Adapter



Deep Well Adapter



96 Flat Bottom Adapter

Software Control

The Opentrons® Heater-Shaker GEN1 is fully programmable in Protocol Designer and the Python Protocol API. The Python API additionally allows for other protocol steps to be performed in parallel while the Heater-Shaker is active. See non-blocking commands in the API documentation for details on adding parallel steps to protocols.

Outside of protocols, the Opentrons® App can display the current status of the module and can directly control the heater, shaker, and labware latch.

Specifications

Dimensions	152 × 90 × 82 mm (L/W/H)
Weight	1.34 kg
Module power input	36 VDC, 6.1 A
Power adapter input	100-240 VAC, 50/60 Hz
Mains supply voltage fluctuation	±10%
Overvoltage	Category II
Power consumption	Idle: 3 W Typical • Shaking: 4–11 W • Heating: 10–30 W • Heating and shaking: 10–40 W Maximum: 125–130 W
Environmental conditions	Indoor use only
Ambient temperature	20-25 °C
Relative humidity	Up to 80%, non-condensing
Altitude	Up to 2,000 m above sea level
Pollution degree	2

Magnetic Block

Features

The Magnetic Block is a magnetic 96-well plate holder. Magnetic blocks are used in protocols that rely on magnetism to pull particles out of suspension and retain them in well plates during wash, rinse, or other elution procedures. For example, automated NGS preparation; purifying genomic and mitochondrial DNA, RNA, or proteins; and other extraction procedures are all use cases that can involve magnetic blocks.



Magnetic Components

The Magnetic Block is unpowered, does not contain any electronic components, and does not move magnetic beads up or down in solution. The wells consist of 96 high-strength neodymium ring magnets fixed to a spring-loaded bed, which helps maintain tolerances between the block and pipettes while running automated protocols.

Software Control

The Magnetic Block is fully programmable in Protocol Designer and the Python Protocol API.

Outside of protocols, however, the touchscreen and the Opentrons® App are not aware of and cannot display the current status of the Magnetic Block. This is an unpowered module. It does not contain electronic or mechanical components that can communicate with the AAW $^{\text{TM}}$ robot. It is 'controlled' via protocols that use the AAW $^{\text{TM}}$ Flex Gripper to add and remove labware from this module.

Specifications

Dimensions	136 × 94 × 45 mm (L/W/H)
Weight	1.13 kg
Module power	None, module is unpowered
Magnet grade	N52 neodymium
Environmental conditions	Indoor use only
Ambient temperature	20-25 °C
Relative humidity	30-80%, non-condensing
Altitude	Up to 2000 m above sea level
Pollution degree	2

Temperature Module

Features

Heating and Cooling

The Opentrons® Temperature Module is a hot and cold plate module. It is often used in protocols that require heating, cooling, or temperature changes. The module can reach and maintain temperatures ranging from 4 °C to 95 °C within minutes, depending on the module's configuration and contents.



Thermal Blocks

To hold labware at temperature, the module uses aluminum thermal blocks. The module comes with 24-well and 96-well thermal blocks. The Temperature Module caddy comes with a deep well block and a flat bottom block designed for use with the AAW $^{\text{TM}}$ Flex Gripper. The blocks hold 1.5 mL and 2.0 mL tubes, 96-well PCR plates, PCR strips, deep well plates, and flat bottom plates.



24-Well Thermal Block



96-Well Thermal Block



Deep Well Thermal Block



Flat Bottom Thermal Block

Software Control

The Temperature Module is fully programmable in Protocol Designer and the Python Protocol API.

Outside of protocols, the Opentrons® App can display the current status of the Temperature Module and can directly control the temperature of the surface plate.

Specifications

Dimensions	194 × 90 × 84 mm (L/W/H)
Weight	1.5 kg
Module power	Input: 100-240 VAC, 50/60 Hz, 4.0 A Output: 36 VDC, 6.1 A, 219.6 W max
Environmental conditions	Indoor use only
Ambient temperature	<22 °C (recommended for optimal cooling)
Relative humidity	Up to 60%, non-condensing
Altitude	Up to 2000 m above sea level
Pollution degree	2

Opentrons® Thermocycler Module GEN2

Features

The Opentrons Flex™ Thermocycler Module GEN2 is a fully automated on-deck thermocycler, providing hands-free PCR in a 96-well plate format. Its heated lid and disposable seal fit tightly over the plate, ensuring efficient sample heating and minimal evaporation.

Heating and Cooling

The Thermocycler's block can heat and cool, and its lid can heat, with the following temperature profile:

- Thermal block temperature range: 4-99 °C
- Thermal block maximum heating ramp rate:
 4.25 °C/s from GEN2 ambient to 95 °C
- Thermal block maximum cooling ramp rate:
 2.0 °C/s from 95 °C to ambient
- Lid temperature range: 37-110 °C
 Lid temperature accuracy: ±1 °C

The automated lid can be opened or closed as needed during protocol execution.



The Thermocycler can execute profiles: automatically cycling through a sequence of block temperatures to perform heat-sensitive reactions.

Rubber Automation Seals

The Thermocycler comes with rubber automation seals to help reduce evaporation. Each seal must be sterilized before use and can be used for several runs. For additional seals, see Product Ordering on page 103.

Software Control

The Thermocycler is fully programmable in Protocol Designer and the Python Protocol API.

Outside of protocols, the Opentrons® App can display the current status of the Thermocycler and can directly control the block temperature, lid temperature, and lid position.

Specifications

Dimensions (lid open)	244.95 × 172 × 310.1 mm (L/W/H)
Dimensions (lid closed)	244.95 × 172 × 170.35 mm (L/W/H)
Weight (including rear duct)	8.4 kg
Power adapter voltage	100-240 V at 50/60 Hz
Power adapter current	8.5-5 A
Overvoltage	Category II
Environmental conditions	Indoor use only
Ambient temperature	20-25 °C (ideal); 2-40 °C (acceptable)
Relative humidity	30-80%, non-condensing
Altitude	Up to 2000 m above sea level
Ventilation requirements	At least 20 cm/8 inches between the unit and a wall



Labware

See the Labware section of the <u>Millipore® Protocol Library</u>, or the <u>Opentrons® Labware Library</u> for the latest listings.

Labware Concepts

Labware encompasses more than just the objects placed on the deck and used in a protocol. In both of the lab library links above provide labware details including items, data that defines each piece of labware, and custom labware.

Labware as Hardware

Everything in the Labware Library can used by default with AAW^{TM} Workstation. These are durable components and consumable items that are worked with, reused, or discarded while running a protocol. The AAW^{TM} robot is programed how to work with everything in the library automatically.

Labware as Data

Labware information is stored in Javascript Object Notation (JSON) files with .json file extensions. A JSON file includes spatial dimensions (length, width, height), volumetric capacity (μ L, mL), and other metrics that define surface features, their shapes, and locations. When running a protocol, the JSON files tell the robot what labware is on the deck and how to work with it.

Custom Labware

Custom labware is labware that is not included in the Labware Library or is labware created by the Custom Labware Creator. However, sometimes the idea of custom labware comes burdened by notions of complexity, expense, or difficulty. Custom labware is not hard to understand or create. Let's take a moment to unpack the concept of custom labware.

The Opentrons® Labware Library includes 96-well plates (200 μ L) from many common manufacturers, but not all manufacturers. Only listed labware has been pre-defined in our Labware Library. The differences among these ubiquitous lab items are minor, but even minor differences in labware dimensions can have a drastic impact on the success of a protocol run. For this reason, it's important to have an accurate labware definition for each labware in the protocol.

The AAW™ Workstation can work with other basic labware items or something unique, but the item's characteristics must be defined in a labware definition JSON file and imported into the Opentrons® App. See <u>Custom Labware Definitions on page 55</u>.

Reservoirs

The AAW™ Workstation works by default with the single-well and multi-well reservoirs listed below. The reservoirs listed below are automation-ready, right out of the box. Reservoir information is also available in the Labware Library.

Single-Well Reservoirs

Manufacturer	Specifications	API Load Name
Agilent	290 mL V bottom	agilient 1 reservoir 290ml
Axygen	90 mL Flat bottom	axygen 1 reservoir 90ml
NEST	195 mL Flat bottom	nest 1 reservoir 195ml
	290 mL V bottom	nest 1_ reservoir_290ml



Multi-Well Reservoirs

Manufacturer	Specifications	API Load Name
NEST	12 wells 15 mL/well V bottom	nest 12 reservoir 15ml
USA Scientific	12 wells 22 mL/well V bottom	usascientific 12 reservoir 22ml



Reservoirs and API Definitions

The Labware Library defines the characteristics of the reservoirs listed above in separate JSON files. The robot and the Python API rely on these JSON definitions to work with labware used in protocols. For example, when working with the API, the **ProtocolContext.load_labware** function accepts these labware names as valid parameters in the code. Linked API load names connect to the reservoir labware definitions in the <u>GitHub repository</u>.

Custom Reservoir Labware

Custom labware can be defined with the Labware Creator. A custom definition combines all the dimensions, metadata, shapes, volumetric capacity, and other information in a JSON file. The AAW^{TM} Workstation needs this information to understand how to work with custom labware. See <u>Custom Labware Definitions on page 55</u>.

Well Plates

The AAW™ Workstation works by default with well plates listed below. The plates listed below are automation-ready, right out of the box. Plate information is also available in the Labware Library.





6-Well Plates

Manufacturer	Specifications	API Load Name
Corning	• 6 wells	corning 6 wellplate 16.8ml flat
	• 16.8 mL/well	
	 Circular wells, flat bottom 	

12-Well Plates

Manufacturer	Specifications	API Load Name
Corning	• 12 wells	corning 12 wellplate 6.9ml flat
	• 6.9 mL/well	
	 Circular wells, flat bottom 	

24-Well Plates

Manufacturer	Specifications	API Load Name
Corning	• 24 wells	corning 24 wellplate 3.4ml flat
	• 3.4 mL/well	
	 Circular wells, flat bottom 	

48-Well Plates

Manufacturer	Specifications	API Load Name
Corning	• 48 wells	corning_48_wellplate_1.6ml_flat
	• 1.6 mL/well	
	 Circular wells, flat bottom 	

96-Well Plates

Manufacturer	Specifications	API Load Name
Bio-Rad	• 96 wells	biorad_96_wellplate_200ul_pcr
	• 200 μL/well	
	 Circular wells, V bottom 	
Corning	• 96 wells	corning 96 wellplate 360ul flat
	• 360 μL/well	
	 Circular wells, flat bottom 	
NEST	• 96 wells	nest 96 wellplate 100ul pcr full skirt
	• 100 μL/well	
	 Circular wells, V bottom 	
	PCR full skirt	
	• 96 wells	nest 96 wellplate 200ul flat
	• 200 μL/well	
	 Circular wells, flat bottom 	
	• 96 deep wells	nest_96_wellplate_2ml_deep
	• 2000 μL/well	
	 Square wells, V bottom 	
Opentrons	 Tough 96 wells 	opentrons 96 wellplate 200ul pcr full skirt
	• 200 μL/well	SKIIL
	 Circular wells, V bottom 	
	PCR full skirt	
Thermo Scientific	Nunc 96 deep wells	thermoscientificnunc_96_wellplate_ 1300ul
	• 1300 μL/well	150001
	Circular wells, U bottom	the constant of the constant o
	Nunc 96 deep wells	thermoscientificnunc 96 wellplate 2000ul
	• 2000 µL/well	
LICA Colombific	Circular wells, U bottom	venezientifie OC wellplate 2 4ml deer
USA Scientific	• 96 deep wells	usascientific 96 wellplate 2.4ml deep
	• 2.4 mL/well	
	 Square wells, U bottom 	

384-Well Plates

Manufacturer	Specifications	API Load Name
Applied Biosystems	• 384 wells	appliedbiosystemsmicroamp_384_
	• 40 μL/well	wellplate_40ul
	 Circular wells, V bottom 	
Bio-Rad	• 384 wells	biorad 384 wellplate 50ul
	• 50 μL/well	
	 Circular wells, V bottom 	
Corning	• 384 wells	corning_384_wellplate_112ul_flat
	• 112 μL/well	
	• Square wells, flat bottom	

Well Plate Adapters

The aluminum plates listed below are thermal adapters for the Opentrons® Heater-Shaker GEN1 module. These standalone adapter definitions can be used to load AAW^{TM} Workstation verified or custom labware on top of the Heater-Shaker.

Adapter Type	API Load Name
Universal Flat Adapter	opentrons universal flat adapter
Flat Bottom Plate Adapter	opentrons 96 flat bottom adapter
PCR Adapter	opentrons 96 pcr adapter
Deep Well Adapter	opentrons 96 deep well adapter

The adapter and labware can both be loaded with a single definition. The Labware Library includes several pre-configured thermal adapter and labware combinations that make the Heater-Shaker ready to use, right out of the box.

Adapter/Labware Combination	API Load Name
Universal Flat Adapter with NEST™ Deep Well Plate 2 mL	opentrons 96 deep well adapter nest wellplate 2ml deep
Universal Flat Adapter with Corning® 384 Well Plate 112 µL Flat	opentrons universal flat adapter corning 384 wellplate 112ul flat
Flat Bottom Plate Adapter with NEST™ 96-Well Plate 200 µL Flat	opentrons 96 flat bottom adapter nest wellplate 200ul flat
PCR Adapter with NEST™ 96-Well Plate 100 µL Full Skirt	opentrons 96 pcr adapter nest wellplate 100ul pcr full skirt

See Product Ordering on page 103.

Well Plates and API Definitions

The Labware Library defines the characteristics of the well plates listed above in separate JSON files. The AAW™ Workstation and the Python API rely on these JSON definitions to work with labware used by protocols. For example, when working with the API, the ProtocolContext.load_labware function accepts these labware names as valid parameters in the code. Linked API Load Names connect to the well plate labware definitions in the <u>GitHub repository</u>.

Custom Well Plate Labware

Try using the Labware Creator to make a custom labware definition if a well plate is not listed. A custom definition combines all the dimensions, metadata, shapes, volumetric capacity, and other information in a JSON file. The AAW™ Workstation reads this information to understand how to work with custom labware. See <u>Custom Labware Definitions on page 55</u>.

Pipette Tips and Tip Racks

The AAW $^{\text{TM}}$ Workstation uses 50 µL, 200 µL, and 1000 µL pipettes. These clear, non-conducting polypropylene tips are available with or without filters. They are packaged sterile in racks of 96 tips and are free of DNase, RNase, protease, pyrogens, human DNA, endotoxins, and PCR inhibitors. Racks also include lot numbers and expiration dates.

Flex pipette tips work with the AAWTM Workstation 50 μ L and 1000 μ L pipettes in the 1-, 8-, and 96-channel configurations. The tip should match the pipette's rated capacity.

Tip Racks

Unfiltered and filtered tips are bundled into a rack that consists of a reusable base plate, a mid-plate that holds 96 tips, and a lid.

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Volume	API Load Name
50 μL	Unfiltered: opentrons flex 96 tiprack 50ul Filtered: opentrons flex 96 filtertiprack 50ul
200 μL	Unfiltered: opentrons flex 96 tiprack 200ul Filtered: opentrons flex 96 filtertiprack 200ul
1000 μL	Unfiltered: opentrons flex 96 tiprack 1000ul Filtered: opentrons flex 96 filtertiprack 1000ul

To help with identification, the tip rack mid-plates are color coded based on tip size:

50 μL: magenta
 200 μL: yellow
 1000 μL: blue

When ordering or reordering, tips and racks come in two different packaged configurations:



- Racks: Consist of separately shrink-wrapped tip racks (base plate, mid-plate with tips, and lid). Racked configurations are best when cleanliness is paramount, to avoid cross-contamination, or when protocols don't allow for base plate or component reuse.
- Refills: Consist of one complete tip rack (base plate, mid plate with tips, and lid) and individual tip containers. Refill configurations are best when protocols allow for base plate or component reuse.

See Product Ordering on page 103

Tip-Pipette Compatibility

Flex pipette tips are designed for the AAW^{TM} Workstation pipettes. Other industry-standard tips may work, but this is not recommended. To ensure optimum performance, only use AAW^{TM} pipettes with Flex pipette tips.

Tip Rack Adapter

The 96-channel pipette requires an adapter to attach a full rack of tips properly. During the attachment procedure, the pipette moves over the adapter, lowers itself onto the mounting pins, and pulls tips onto the pipettes by lifting the adapter and tip rack.

Note: Only use the tip rack adapter when picking up a full rack of tips at once. Place tip racks directly on the deck when picking up fewer tips.

Warning: Pinch point hazard. Keep hands away from the tip rack adapter while the pipette is attaching pipette tips.



Adapter type

API Load Name

Opentrons® 96-Channel Tip Rack Adapter opentrons flex 96 tiprack adapter

The tip rack adapter is compatible with the AAW™ Flex Gripper. Use the gripper to place fresh tip racks on the adapter or to pick up and move used tip racks into the waste chute.

Tubes and Tube Racks

The 4-in-1 Tube Rack system works with the AAW™ Workstation by default and is automation-ready. More information is also available in the Labware Library.

Tube and Rack Combinations

The 4-in-1 tube rack supports a wide variety of tube sizes, singly or in different size (volume) combinations. These include:

- 6-tube rack for 50 mL tubes (6 x 50 mL)
- 10-tube combination rack for four 50 mL tubes and six 15 mL tubes (4 x 50 mL, 6 x 15 mL)
- 15-tube rack for 15 mL tubes (15 x 15 mL)
- 24-tube rack for 0.5 mL, 1.5 mL, or 2 mL tubes (24 x 0.5 mL, 1.5 mL, 2 mL)

Note: All tubes are cylindrical with V-shaped (conical) bottoms unless otherwise indicated.



6-Tube Racks

Tube type	API Load Name
6 Falcon™ 50 mL	opentrons 6 tuberack falcon 50ml conical
6 NEST™ 50 mL	opentrons 6 tuberack nest 50ml conical

10-Tube Racks

Tube type	API Load Name
4 Falcon™ 50 mL	opentrons 10 tuberack falcon 4x50ml 6x15ml conical
6 Falcon™ 15 mL	
4 NEST™ 50 mL	opentrons 10 tuberack nest 4x50ml 6x15ml conical
6 NEST™ 15 mL	

15-Tube Racks

Tube type	API Load Name
15 Falcon™ 15 mL	opentrons 15 tuberack falcon 15ml conical
15 NEST™ 15 mL	opentrons 15 tuberack nest 15ml conical

24-Tube Racks

Tube type	API Load Name
24 Eppendorf [™] Safe-Lock 1.5 mL	opentrons 24_tuberack_eppendorf_1.5ml_safelock_snapcap
24 Eppendorf™ Safe-Lock 2 mL, U-shaped bottom	opentrons 24 tuberack eppendorf 2ml safelock snapcap
24 generic 2 mL screw cap	opentrons 24_tuberack_generic_2ml_screwcap
24 NEST™ 0.5 mL screw cap	opentrons 24 tuberack nest 0.5ml screwcap
24 NEST™ 1.5 mL screw cap	opentrons 24_tuberack_nest_1.5ml_screwcap
24 NEST™ 1.5 mL snap cap	opentrons 24 tuberack nest 1.5ml snapcap
24 NEST™ 2 mL screw cap	opentrons 24 tuberack nest 2ml screwcap
24 NEST™ 2 mL snap cap, U-shaped bottom	opentrons 24 tuberack nest 2ml snapcap

Tube Rack API Definitions

The Labware Library defines the characteristics of the tube racks listed above in separate JSON files. The AAW™ Workstation and the Python API rely on these JSON definitions to work with labware used by protocols. For example, when working with the API, the ProtocolContext.load_labware function accepts these labware names as valid parameters in the code. Linked API load names connect to the tube rack labware definitions in the GitHub repository.

Custom Tube Rack Labware

Try creating a custom labware definition using the Labware Creator if a tube and rack combination is not listed. A custom definition combines all the dimensions, metadata, shapes, volumetric capacity, and other information in a JSON file. The AAW™ Workstation reads this information to understand how to work with custom labware. See <u>Custom Labware Definitions on page 55</u>.

Aluminum Blocks

Aluminum blocks ship with the Temperature Module GEN2 and can be purchased separately as a three-piece set. The set includes a flat bottom plate, a 24-well block, and a 96-well block.

The AAW™ Workstation uses aluminum blocks to hold sample tubes and well plates on the Temperature Module or directly on the deck. When used with the Temperature Module, the aluminum blocks can keep sample tubes, PCR strips, or plates at a constant temperature between 4 °C and 95 °C.

Flat Bottom Plate

The flat bottom plate ships with the Temperature Module's caddy and is compatible with various ANSI/SLAS standard well plates. This flat plate differs from the plate that ships with the Temperature Module itself or the separate three-piece set. It features a wider working surface and chamfered corner clips. These features help improve the performance of the AAW™ Flex Gripper when moving labware onto or off of the plate.



24-Well Aluminum Block

The 24-well block is used with individual sample vials. For example, it accepts sample vials that:

- Have V-shaped or U-shaped bottoms.
- Secure contents with snap cap or screw cap closures.
- Hold liquid in capacities of 0.5 mL, 1.5 mL, and 2 mL.



The 96-well block supports a wide variety of well plate types. For example, it accepts well plates that are:

- From major well-plate manufacturers like Bio-Rad and NEST.
- Designed with V-shaped bottoms, U-shaped bottoms, or flat bottoms.
- Designed with 100 μL or 200 μL wells.

It is also compatible with generic PCR strips.



Standalone Adapters

Thermal block	API Load Name
AAW™ flat bottom plate	opentrons_aluminum_flat_bottom_plate
24-well aluminum block	See <u>Aluminum Block Labware Combinations on page 54</u> .
96-well aluminum block	opentrons_96_well_aluminum_block

Aluminum Block Labware Combinations

The Labware Library supports the following block, vial, and well plate combinations, which are also defined in separate JSON labware definition files. The AAW™ Workstation and the Python API rely on these JSON definitions to work with labware used by the protocols. For example, when working with the API, the ProtocolContext.load_labware function accepts these labware names as valid parameters in your code. The tables below list the default block/container combinations and related API load names. Links connect to corresponding JSON definitions in the GitHub repository.

Note: All tubes have V-shaped bottoms unless otherwise indicated.

24-Well Aluminum Block Labware Combinations

24-well block contents	API Load Name
Generic 2 mL screw cap	opentrons 24_aluminumblock_generic_2ml_screwcap
NEST™ 0.5 mL screw cap	opentrons 24 aluminumblock nest 0.5ml screwcap
NEST™ 1.5 mL screw cap	opentrons 24_aluminumblock_nest_1.5ml_screwcap
NEST™ 1.5 mL snap cap	opentrons 24 aluminumblock nest 1.5ml snapcap
NEST™ 2 mL screw cap	opentrons 24_aluminumblock_nest_2ml_screwcap
NEST™ 2 mL snap cap, U-shaped bottom	opentrons 24_aluminumblock_nest_2ml_snapcap

96-Well Aluminum Block Labware Combinations

96-well block contents	API Load Name
Bio-Rad® well plate 200 μL	opentrons_96_aluminumblock_biorad_wellplate_200uL
Generic PCR strip 200 μL	opentrons 96 aluminumblock generic pcr strip 200uL
NEST™ well plate 100 μL	opentrons_96_aluminumblock_nest_wellplate_100uL

Labware and the AAW™ Flex Gripper

Although the AAW™ Workstation works with all the inventory in the Labware Library, the Flex Gripper is compatible with specific labware items only. Currently, the Gripper is optimized for use with the following labware items.

Labware category	Brands
Deep Well Plates	• NEST™ 96-Well Deep Well Plate 2 mL
Fully Skirted 96 Well Plates	 Opentrons® Tough 96-Well PCR Plate Full Skirt 200 μL NEST™ 96-Well Plate 200 μL Flat
Tip Racks (unfiltered and filtered tips)	 Opentrons® 96-Channel Tip Rack 50 μL Opentrons® 96-Channel Tip Rack 200 μL Opentrons® 96-Channel Tip Rack 1000 μL

Note: For best results, use the AAW[™] Flex Gripper only with the labware listed above. The AAW[™] Flex Gripper may work with other ANSI/SLAS automation compliant labware, but this is not recommended.

Custom Labware Definitions

Custom labware is labware that's not listed in the Labware Library. You can use other common or unique labware items with the AAW™ Workstation by accurately measuring and recording the characteristics of that object and saving that data in a JSON file. When imported into the app, the AAW™ Workstation and the API uses that JSON data to interact with labware.

Creating Custom Labware Definitions

These features accommodate different skill levels and ways of working.

Custom Labware Creator

The Custom Labware Creator is a no-code, web-based tool that uses a graphical interface to help create a labware definition file. Labware Creator produces a JSON labware definition file that imports into the Opentrons® App. After that, custom labware is available to the AAW™ Workstation and the Python API.

Custom Labware Service

Contact <u>SigmaAldrich.com/TechService</u> if the labware isn't available in the library, if you can't create a definitions, or because a custom item includes different shapes, sizes, or other irregularities described below.

Custom Labware the Labware Creator Can Define	Custom Labware that will Need Assistance to Define					
lackim Wells and tubes are uniform and identical.	✓ Wells and tube shapes vary.					
All rows are evenly spaced (the space between rows is equal).	Rows are not evenly spaced.					
All columns are evenly spaced (the space between columns is equal).	✓ Columns are not evenly spaced.					
Fits perfectly in one deck slot.	✓ Smaller than one deck slot (requires adapter) or spans multiple deck slots.					

Regular All columns are evenly spaced and all rows are evenly spaced. Columns do not need to have the same spacing as rows. **▼** Regular The grid does not have to be in the center of labware. **Irregular** Rows are evenly spaced but columns are not evenly spaced. Irregular Irregular Columns/rows are evenly spaced but wells are not identical. **Irregular** 0000 There is more than one grid. 0000 0000 0000 0000

Our labware team will work to understand the lab needs and design the custom labware definitions. See <u>Custom Labware Definitions on page 55</u> and <u>Custom Labware Service on page 55</u>.

Python API

Examples

Custom labware cannot be created with with our API. However, it can be created with available API methods. Custom labware must be defined first, and imported it into the Opentrons® App. Once the labware has been added to the Opentrons® App, it can be accessed by the Python API and the robot. See <u>Custom Labware Definitions on page 55</u>. For information about writing protocol scripts with the API, see <u>Python Protocol API on page 68</u>.

JSON labware schema

A JSON file is the blueprint for standard and custom labware. This file contains and organizes labware data according to the design specifications set by the default schema.

A schema is a framework for organizing data. It sets the rules about what information is required or optional and how it's organized in the JSON file. The following table lists and defines the items in the AAW^{TM} labware schema.

Property	Data type	Definition					
schemaVersion	Number	Schema version used by a labware. The current version is 2.					
version	Integer	An incrementing integer that identifies the labware version. Minimum version is 1.					
namespace	String	See safeString in the <u>JSON Labware Definitions on page 60</u> .					
metadata	Object	Properties used for search and display. Accepts only:					
		• displayName (String): An easy-to-remember labware name.					
		 displayCategory: Labels used in the UI to categorize labware. See displayCategory in the <u>JSON Labware Definitions on page 60</u>. 					
		\bullet displayVolumeUnits (String): Labels used in the UI to indicate volume. Must be either $\mu L,\ mL,\ or\ L.$					
brand	Object	Information about the labware manufacturer or those products the labware is compatible with.					
parameters	Object	Internal parameters that describe labware characteristics. Accepts only:					
		 format (String): Determines labware compatibility with multichannel pipettes. Must be one of 96Standard, 384Standard, trough, irregular, or trash. 					
		• quirks (Array): Strings describing labware behavior.					
		 isTiprack (Boolean): Indicates if labware is a tip rack (true) or not (false). 					
		 tipLength (Number): Required if labware is a tip rack. Specifies tip length (in mm), from top to bottom, as indicated in technical drawings or as measured with calipers. 					
		• tipOverlap (Number): Required if labware is a tip rack. Specifies how far tips on a tip rack are expected to overlap with the pipette's nozzle. Defined as tip length minus the distance between the bottom of the pipette and the bottom of the tip. The robot's calibration process may fine-tune this estimate.					
		 loadName: Name used to reference a labware definition (e.g., opentrons_flex_96_tiprack_50_ul). 					
		• isMagneticModuleCompatible (Boolean): Indicates if labware is compatible with the Magnetic Module.					
		 magneticModuleEngageHeight: How far the Magnetic Module will move its magnets when used with this labware. See positiveNumber in the JSON definitions section on the next page. 					
ordering	Array	An array that tracks how wells should be ordered on a piece of labware. See the $\underline{example}$.					

Property	Data type	Definition					
cornerOffset FromSlot	Object	Used for labware that spans multiple deck slots. Offset is the distance from the left-front-bottom corner of the slot to the left-front-bottom corner of the labware bounding box. Accepts only:					
		• x (number)					
		• y (number)					
		• z (number)					
		For labware that does not span multiple slots, these values should be zer See positiveNumber in the JSON Labware Definitions on page 60 .					
dimensions	Object	Outer dimensions (in mm) of a piece of labware. Accepts only:					
		• xDimension (length)					
		• yDimension (width)					
		• zDimension (height)					
		<u>Example</u>					
wells	Object	An unordered object of well objects, including position and dimensions.					
		Each well object's key is the well's coordinates, which must be an uppercase letter followed by a number, e.g., A1, B1, H12.					
		Each well object accepts the following properties:					
		 depth (Number): The distance (in mm) between the top and bottom of the well. For tip racks, depth is ignored in favor of tipLength, but the values should match. 					
		• x (Number): Location of the center-bottom of a well in reference to the left of the labware.					
		 y (Number): Location of the center-bottom of a well in reference to the front of the labware. 					
		• z (Number): Location of the center-bottom of a well in reference to the bottom of the labware.					
		\bullet total LiquidVolume (Number): Total well, tube, or tip volume in $\mu L.$					
		 xDimension (Number): Length of a rectangular well. 					
		 yDimension (Number): Width of a rectangular well. 					
		 diameter (Number): Diameter of a circular well. 					
		 shape (String): Either rectangular or circular. If rectangular, specify xDimension and yDimension. If circular, specify diameter. 					
		See circular well <u>example</u> . See rectangular well <u>example</u> .					
		For dimension, depth, and volume, see positiveNumber in the JSON Labware Definitions on page 60.					

Property	Data type	Definition			
groups	Array	Logical well groupings for metadata and display purposes. Changes in groups do not affect protocol execution. Each item in the array accepts:			
		 wells (Array): An array of wells (e.g., ["A1","B1","C1"]) that share the same metadata. Array elements are strings. 			
		• metadata (Object): Metadata specific to a grid of wells. Accepts only:			
		 displayName (String): Human-readable name for the well group. 			
		 displayCategory: Labels used to categorize well groups. See displayCategory in the JSON definitions section below. 			
		 wellBottomShape (String): Bottom shape of a well. Available shapes are flat, u, or v only. 			
		 brand: Brand information for the well group. See brandData in the <u>JSON Labware Definitions on page 60</u>. 			
allowedRoles Array		Defines an item's role or purpose. If the allowedRoles field is missing from a definition, an item is treated as labware. Possible array items are only the following strings:			
		labware (standard labware items)			
		adapter (items designed to hold labware)			
		 fixture (items that are affixed to the deck) 			
		 maintenance (items not used in normal protocol runs) 			
stackingOffset WithLabware	Object	For labware that can stack on top of another piece of labware. Used to determine z-height (labware z height + adapter z height - overlap). See coordinates in the JSON Labware Definitions on page 60.			
stackingOffset WithModule	Object	For labware that can stack on top of a module. Used to determine z-height (module labware offset $z + labware z - overlap$). See coordinates in the JSON Labware Definitions on page 60.			
gripperOffsets	Object	Offsets added when calculating the coordinates the gripper should go to when picking up or dropping other labware on this labware. Includes a default object that includes two properties:			
		 pickUpOffset: Offset added to calculate the pick-up coordinates of labware placed on this labware. 			
		 dropOffset: Offset added to calculate the drop-off coordinates of labware placed on this labware. 			
		See coordinates in the <u>JSON Labware Definitions on page 60</u> .			
gripForce	Number	Measured in newtons, this is the force which the gripper uses to grasp labware. Recommended values are between 5 and 16.			
gripHeight FromLabware Botton	Number m	Recommended z-axis height, from the labware bottom to the center of the gripper pads.			

JSON Labware Definitions

Property	Data type	Definition
positiveNumber	Number	Minimum: 0
brandData	Object	Information about branded items. Accepts only:
		 brand (String): Brand/manufacturer's name
		 brandId (Array): OEM part numbers or IDs
		• links (Array): Manufacturer URLs. Array items are strings.
displayCategory	String	Must be one of:
		• tipRack
		• tubeRack
		• reservoir
		• trash
		• wellPlate
		aluminumBlock
		• adapter
		• other
safeString	String	A string safe to use for load names and namespaces. Lowercase letters, numerals, periods, and underscores only.
coordinates	Object	Coordinates that specify a distance or position along the x -, y -, and z -axes. Accepts only:
		• x (number)
		• y (number)
		• z (number)

Protocol Development

The AAW™ Workstation can run a wide variety of automated protocols, for tasks such as PCR, NGS, ELISA, and many more. You can run fully built and verified protocols, edit community protocols to suit lab needs, or design protocols from the beginning, with or without writing code.

Pre-Made Protocols

Millipore® Protocol Library

The Millipore® Protocol Library hosts protocols authored by Opentrons Labs, LLC or members of the Opentrons® community. Only the AAW™ Workstation unlocks access to the Millipore® Protocol Library. To find a protocol that fits your target application, use the search field at the top of the Protocol Library homepage.

You can also browse protocols by categories, like DNA/RNA, cell biology, cell and tissue culture, proteins, commercial assay kits, or molecular biology. There's even a category for protocols that create art by pipetting! Take some time to check out the protocols in both libraries. Understanding what's available is a great way to learn about the features and capabilities of your robot before moving on to using real samples and reagents.

Searching for Protocols

The Protocol Library search returns results as you type. Select a result from the search list or click **View All Results** to go to the full results page, which shows more details about each protocol and allows filtering based on several criteria.

Each protocol card will show:

Item	Description					
Protocol name	The name of the protocol.					
Verification	Badges indicate if the protocol is verified by AAW^{TM} , a third-party manufacturer, or members of the community.					
Time estimate	Approximately how long the protocol takes to run.					
Description	A short summary of what the protocol does.					
Robot model	Which AAW^{TM} robots the protocol is compatible with.					
Protocol editability	JSON protocols are editable in Protocol Designer, with no coding required. Python protocols are editable in any text editor, using the Python Protocol API.					
Modules	Any hardware modules that are required.					

In addition to these categories, in the sidebar you can filter results by:

- Pipettes: Which pipettes the protocol uses (you can usually change a protocol's pipettes, but it may affect the run time and the number of tips consumed).
- Categories: Target applications, like DNA/RNA, cell biology, proteins, etc.
- Protocol version: Show or hide older versions of protocols.

Protocol Details

Click on a protocol to go to its detail page, which provides even more information. In addition to what is shown in search, here you can see:

- Supporting data: Additional data, explanations, or links to outside sources provided by the protocol author.
- What you'll need: A complete list of all equipment needed for the protocol, including the robot, modules, labware, pipettes, and third-party kits.
- Protocol steps: A list of steps written by the protocol author, as well as a visual deck map and list of liquids specified in the protocol file.

The details page also provides basic instructions for downloading and running the protocol. For more information on importing a protocol to the Opentrons® App and setting up a run, see <u>Transferring</u> Protocols to the AAW™ Workstation on page 86.

Custom Protocol Development Service

Custom Protocol Development Services can be enlisted for a separate fee if a protocol is not already included in the Protocol Library. This comprehensive protocol development service can take approximately three (3) months. As part of the service, a field applications scientist will:

- Develop the Python code for one (1) kit or workflow
- Deliver the Python code and protocol summary
- Support optimization of your protocol within one month of initial delivery. (Optimization includes protocol steps and liquid classes)

Note: The Custom Protocol Development Service only writes Python protocols that control the Opentrons Flex[™] robot or AAW[™] Workstation. It does not cover controlling the robot with code in other languages, nor does it cover controlling third-party hardware.

Protocol Request Guidelines

Describing your protocol in detail enables our field applications scientists to accurately code the automation that you need. Consider your protocol's requirements, including:

- Hardware (pipettes, gripper, modules, fixtures).
- Labware (verified, other standard, or custom).

Also consider special cases that apply to your protocol, like:

- Liquids that are volatile, viscous, or otherwise behave differently than water.
- Conservation of expensive reagents.
- Sterility and cross-contamination.
- Advanced pipetting techniques like air-gapping, high or low flow rate, or pipetting at specific locations within wells.

To explain the movements the robot will make in executing the protocol, start with your initial deck state. Where should modules, labware, and trash containers be located? Which liquids will be in which labware, and in what quantities? Use the coordinate systems printed on the AAW™ Workstation deck and on standard labware to describe these locations.

Next, give step-by-step instructions on how AAWTM Workstation should handle liquids, specifying quantities in microliters (μ L) and giving exact source and destination locations (rows, columns, or individual wells of labware).

In general, following the style of the methods section of an academic paper will help the AAW™ team understand the instructions. Always err on the side of providing extra information.

Custom Protocol Pricing

The Custom Protocol Development service is open to all owners of AAW™ Workstation systems. Pricing is based on complexity of your workflow. Contact <u>SigmaAldrich.com/TechService</u> for pricing information.

Complexity	Description				
Complex	Protocols that require complex Python code and a longer time for protocol optimization. Example applications include NGS, nucleic acid purification, nucleic acid extraction, protein expression, and purification.				
Moderately Complex	Protocols that require moderately complex Python code and time for protocol optimization. Example applications include ELISA, protein crystallization, Bradford Assays or BCA assays, transfection or transformation assays, drug discovery, MALDI spotting, microarray prep, flow cytometry, complex serial dilutions, workflows with multiple variables, and workflows with highly unique plate maps.				
Simple	Protocols that require basic Python code and minimal optimization. Example applications include PCR setup, sample aliquoting, normalization, cherry-picking, CSV file handling, media exchange, and mass spectrometry sample preparation.				
Amendments	Amendments to existing protocols in the Protocol Library only.				

Protocol Designer

The Protocol Designer is a web-based, no-code tool for developing protocols that run on the AAW™ Workstation to perform tasks:

- Aspirate, dispense, transfer, and mix liquids.
- Move labware around the deck with the gripper.
- Operate AAW™ Workstation modules.
- Pause to let you verify progress or access samples.

All work on your protocol takes place within your web browser. When you're done creating or editing your protocol, you need to export it to a JSON file. Then upload that file to a robot and run it, as you would with any protocol.

Protocol Designer Requirements

Currently, the Protocol Designer is only supported for use in Google Chrome and requires an internet connection. Uploading and running JSON protocols on AAW[™] Workstation requires version 7.0.0 or later of the Opentrons® App.

You can't create or modify Python protocol files with Protocol Designer.

Designing a Protocol

Protocols are all about informing the robot what hardware it will use to take specific actions. This process is broken down into three tabs in Protocol Designer:

Icon Tab



The File tab is where you manage protocol files and specify hardware for use in your protocol.



The Liquids tab lets you define samples, reagents, and any other liquids that your robot will handle.



The Design tab is where you specify the initial state of the deck, add steps that the robot will perform, and view the projected outcomes of those steps.

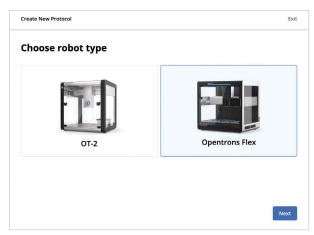
To create a protocol from scratch, start on the File tab, work with the Liquids and Design tabs, and then return to the File tab to export your work. The remainder of this section goes through the protocol creation process in detail.

4. Create a Protocol

Create a protocol in Protocol Designer

When you launch Protocol Designer, open the **File** tab. In the left sidebar, click **Create New** to open the **Create New Protocol** dialog. Tp create a protocol for AAW™ Workstation, click on the image of the **Opentrons Flex** and then click **Next**.

Enter a name for your protocol, which is how it will appear in the Opentrons® App and on the touchscreen. You'll also see your protocol name in the Protocol Designer header while you're working on it. Optionally add a description and author information for your protocol.



Next, Protocol Designer guides you through choosing the hardware used in your protocol:

- Pipettes and what type of tip racks you'll use with them. Every protocol requires at least one pipette.
 - Staging area slots in column 3 (optional).
 - Additional hardware used in your protocol, such as modules, the gripper, or the waste chute. Only modules that are compatible with the AAW™ Workstation are shown.

At any time, you can return to the File tab to rename your protocol, add an author name or description, or change your hardware configuration.

5. Define Liquids

Move on to the Liquids tab to set up samples and reagents. This tab is only for defining types of liquids. You'll indicate the starting positions and amounts of liquids in Part 3, on the Design tab.

Click New Liquid and then enter the name of your liquid and an optional description. You can also choose whether to serialize the liquid, so each well containing that liquid will be numbered on the deck map and in action steps. For example, if your protocol has blood samples, serialization can help you keep them separate in your workflow, while still labeling them all as "blood" and color-coding them the same.

Each type of liquid appears in a different color on the deck map in Protocol Designer, in the Opentrons® App, and on the touchscreen. You can use the default color, pick another preset color, or enter an RGB hex code to set a custom color.

6. Lay Out the Deck

Go to the Design tab to do the final setup step, which is placing labware and liquids on the deck. The main view on this tab is the deck map, which shows everything on the deck down to individual wells — even on 384-well plates.

The deck map starts with the tip racks and modules you chose for your protocol in their default locations. Hover over any open slot and click Add Labware or Adapter to add more tip racks, other types of labware, or adapters. Drag and drop labware to an open slot to move it there, or to an occupied slot to swap the two pieces of labware.

Note: You can't move modules or adapters around the deck map by drag and drop. This is to make it easier to move labware onto or off of a module.

- To change a module's position, return to the File tab and click Edit next to the module name.
- To change an adapter's position, add a new adapter. Then move the labware from the old adapter to the new adapter. Finally, delete the old adapter.

Hover over any labware and click Add Liquids to specify which wells contain which liquid. Clicking on a single well or dragging across a range of wells will reveal a form at the top of the screen. Choose one of the liquids you defined and the volume each well should start with, in μL . For example, if you select the first column on a 96-well plate and specify 100 μL , that will be 800 μL of liquid total (100 $\mu L \times 8$ wells).

7. Add Steps

Click Add Step and choose the type of movement desired.

- · Pipetting steps
 - Transfer: Move liquid from one well or group of wells to another. Specify the source, where liquid will be aspirated from, on the left. Specify the destination, where liquid will be dispensed, on the right. Click either gear icon to change behaviors such as flow rate, tip height, knocking droplets off (touch tip), air gapping, blowout, and more. In the Sterility & Motion section, choose the correct tip-use strategy for your application.
 - Mix: Repeatedly aspirate and dispense liquid within the same well. Choose how much liquid to
 mix with, the number of mixing repetitions, and which wells will be mixed. Like with transfer
 steps, click either gear to change mixing behavior. You can also choose a tip-use strategy for
 mixing. These options are more limited than for transfers, since all liquid returns to its starting
 location when mixing.

· Gripper steps

Move Labware: Control the AAW^{TM} Flex Gripper or move labware around the deck manually. Choose which labware you want to move and its new location. Check the **Use Gripper** box to have the gripper move the labware automatically, or leave it unchecked to have the protocol pause so you can move the labware manually. You need to use the gripper to dispose labware by moving it into the waste chute. You need to move labware manually to move it off the deck (without disposing it).

Module steps

- Heater-Shaker: Control the temperature, shake speed, and labware latch of the Heater-Shaker Module. You can set an optional timer that will pause the protocol for a set period of time after the other actions are completed (heating to high temperatures or waiting for the module to passively cool to a temperature can take a long time).
- Temperature: Set a target temperature or deactivate the Temperature Module.
- Thermocycler: This action has two mutually exclusive sets of options.
 - Change Thermocycler state: Set a block temperature, set a lid temperature, or move the lid.
 - Program a Thermocycler profile: Define a profile, a timed heating and cooling routine that can be automatically repeated. Each step of the profile holds the block at a certain temperature for a certain time. Profiles do not change the temperature of the lid.

• Pause

Prevent the protocol from continuing until one of three criteria is met. Pauses can require user intervention (pressing a button on the touchscreen or in the app), wait for a fixed time, or wait until a module reaches a target temperature. Timed pauses are useful for incubation or letting the Magnetic Block work.

8. Edit Steps

Once you've created a step, preview its effects by hovering over it in the Protocol Timeline. Affected tips and wells will be highlighted, as will the entire labware containing those wells.

Show or hide the details of a step by clicking the disclosure triangle to the right of its name. For liquid handling steps, this will show every discrete aspirate and dispense pair comprising the step. For module steps, this will show the features of the module that the step controls.

Click on the name of a step in the Protocol Timeline to edit it. Shift-click to select a range of steps and enter batch editing mode. If you select only transfer or mix steps, you can change their behavior as a batch. Reorder steps by dragging and dropping them up or down in the Protocol Timeline.

When editing any step, click Notes to change the step name or add a description of what the step does. Custom step names replace their default action descriptions (like "Transfer" and "Temperature") in the Protocol Timeline, making it easier to navigate around your protocol.

9. Export Your Protocol

When your protocol is complete, click Final Deck State to preview how the deck should appear at the end of your protocol. In this view (or when viewing a particular step), you can click on labware and examine the expected quantity of liquid in each well.

To save your work, return to the File tab and click Export to download your protocol as a JSON file. The file will have the name you chose in the Protocol Name field and will have a .json extension. You can find exported protocols in the default download location of your web browser.

To run your protocol, import it into the Opentrons® App. (See <u>Software and Operation on page 72</u> for installing and using the Opentrons® App.) Then either run it from the app or send it to the AAW™ Workstation to run from the touchscreen.

Modifying Existing Protocols

Click **Import** in the File tab to load an existing protocol. Choose any JSON protocol file from the standard system file picker. Once loaded, you can edit any aspect of the protocol, including its name, description, hardware configuration, and steps.

Warning: Importing a protocol will replace any other protocol that you've been working on in Protocol Designer. Be sure to export your work before importing another file, or open Protocol Designer in a second browser tab to work on multiple files at once.

Python Protocol API

Writing protocol scripts in Python gives the most fine-grained control of AAW™ Workstation. The Python Protocol API version 2 is a single Python package that exposes a wide range of liquid handling features on Opentrons Flex™ and AAW™ robots. For an idea of the breadth of the API, check out the full online documentation, which includes topic-based articles as well as a comprehensive reference of all methods and functions contained in the package. We recommend following the Tutorial to become aquainted with a robotic protocol before attempting to build one from the beginning.

Writing and Running Scripts

Python protocols generally follow the same basic structure:

- 1. Importing the AAW™ package.
- 2. Declaring the requirements and metadata in their respective dictionaries.
- 3. Defining a run() function that contains all of the instructions to the robot, including:
 - Pipettes the protocol will use.
 - Locations of modules, labware, and deck fixtures.
 - Liquid types and locations (optional).
 - Commands the system will physically execute (e.g., simple or complex liquid handling commands, module commands, or movement commands).

When running a protocol via the Opentrons® App or the touchscreen, it is not necessary to call the run() function, because the robot software does it automatically.

However, one of the advanced features of the Python API is to control a robot outside of the usual flow for setting up and running a protocol. The AAW™ Workstation runs a Jupyter Notebook server, which can execute discrete blocks of code (called cells), rather than a complete protocol file. When organizing code into cells define a run() function (and then call it) or run commands without one. It is also possible to execute complete protocols in a Jupyter terminal session or when connected to the AAW™ Workstation via Secure Shell (SSH). For more information, see Command-Line Opration over Secure Shell (SSH) on page 89.

Python-Exclusive Features

Certain features are only available in Python protocols, either because they are part of the API or because of the inherent flexibility of Python code.

Python Packages

The Python API supports features not included in Protocol Designer. The Python protocol is a Python script, which means that it can perform any computation that relies on the Python standard libraries or the suite of libraries included in the system software.

Additional Python packages can be installed. Connect to the AAW™ Workstation via SSH and install the package with pip. To avoid analysis errors in the Opentrons® App, install the packages on your computer as well. In the Opentrons® App settings, go to Advanced and click Add override path in the Override Path to Python section. Choose the copy of python on your system that has access to the packages.

OT-2 Protocols

There are hundreds of OT-2 protocols in the Protocol Library. The AAW™ Workstation can perform all the basic actions that the OT-2 can, but OT-2 protocols aren't directly compatible. The OT-2 protocols can be adapted which provides parity across different AAW™ Workstations and Opentrons® robots in the lab, or extend older protocols to take advantage of new features only offered on the AAW™ Workstation.

OT-2 Python Protocols

An OT-2 protocol requires a few changes using the Python Protocol API for it to run on AAW^{TM} Workstations.

Metadata and Requirements

The API requires you to declare that a protocol is designed to run on AAW™ Workstation. Use the robotType key in the new requirements dictionary. Specify an apiLevel of 2.15 or higher and an apiLevel either in the metadata dictionary or the requirements dictionary.

```
from opentrons import protocol_api
requirements = {\robotType': \Flex', \apiLevel': \2.15'}
```

Pipettes and Tip Racks

Flex uses different types of pipettes and tip racks than OT-2, which have their own load names in the API. Choose pipettes of the same capacity or larger (or whatever you've outfitted your Flex with). For example, you could convert an OT-2 protocol that uses a P300 Single-Channel GEN2 pipette and 300 μ L tips to a Flex protocol that uses a Flex 1-Channel 1000 μ L pipette and 1000 μ L tips:

```
# Original OT-2 code

def run(protocol: protocol_api.ProtocolContext):
    tips = protocol.load_labware("opentrons_96_tiprack_300ul", 1)
    left_pipette = protocol.load_instrument(
        "p300_single_gen2", "left", tip_racks=[tips]
```

```
# Modified Flex code

def run(protocol: protocol_api.ProtocolContext):
    tips = protocol.load_labware("opentrons_flex_96_tiprack_1000ul", "D1")
    left_pipette = protocol.load_instrument(
        "flex_1channel_1000", "left", tip_racks[tips]
)
```

The only necessary changes are the new arguments of load_labware() and load_instrument(). Keep in mind that if you use smaller capacity tips than the original protocol, you may need to make further adjustments to avoid running out of tips, and the protocol may take longer to execute.

Deck Slots

The API accepts OT-2 and AAW™ Workstation deck slot names interchangeably. It is good practice to use the coordinate deck slot format in AAW™ protocols (as in the example in the previous subsection), but it's not required. The correspondence between deck slot numbers is as follows:

AAW [™] Workstation or Opentrons Flex [™]	A1	A2	А3	B1	B2	В3	C1	C2	C3	D1	D2	D3	
OT-2	10	11	Trash	7	8	9	4	5	6	1	2	3	

A protocol that calls protocol.load_labware("opentrons_flex_96_tiprack_200ul", "1") would require the tip rack placed in slot D1 on the robot.

Modules

The AAW™ Workstation supports:

- Temperature Module Gen2
- Thermocycler Module Gen2 or Thermocycler Module2
- The Heater-Shaker Module only has one generation, which is also compatible with the older version OT-2.
- For protocols that load magnetic module, magdeck, or magnetic module gen2, see Magnetic Module Protocols on page 71.

OT-2 JSON Protocols

The Protocol Designer cannot convert an OT-2 protocol to a Flex or AAW™ Workstation protocol. You have to choose which robot the protocol will run on when you create it.

Since Flex protocols support nearly all the features of the older OT-2 protocols, you can create a new protocol that performs all the same steps, but is designed to run on the AAW^{TM} Workstation. The simplest way to do this is:

- 1. Launch Protocol Designer and import the OT-2 protocol.
- 2. Open a second browser window and launch Protocol Designer there.
- 3. Create a new AAW™ Workstation protocol in the second browser window.
- 4. Set up the hardware as similarly as possible as the OT-2 hardware. For example, choose pipettes of the same capacity or larger, and choose modules of the same type.
- 5. Replicate the liquid setup and steps from the OT-2 protocol.
- 6. Export your new protocol. Import it into the Opentrons® App and check the run preview to see that it performs the same steps as the OT-2 protocol.

If bigger changes are made and the new configuration differs significantly from the OT-2 configuration, the protocol should be re-verified.

Magnetic Module Protocols

There is no direct analogue of the Magnetic Module. You'll have to use the Magnetic Block and AAW™ Flex Gripper instead. This will require reworking some of your protocol steps, and you should verify that your new protocol design achieves similar results.

Software and Operation

There are multiple ways to control the AAW™ Workstation, depending on lab needs. Most functions can be performed from the touchscreen or a computer running the Opentrons® App. This chapter will focus primarily on touchscreen operation, and will only cover features of the Opentrons® App that are not possible on the touchscreen. It will also outline advanced control features, such as running Python code using the Jupyter Notebook server or from the command line of AAW™ Workstation.

One major difference between touchscreen and app operation is the software's relationship to the robot. The touchscreen is integrated, and therefore only controls the robot that it's physically a part of. In contrast, the Opentrons® App can control any number of AAWTM robots connected to the computer that's running the app. Using both pieces of software is required to set up the AAWTM Workstation and run your first protocol, but it's up to the user to decide the balance of how to control the AAWTM Workstation in your daily workflow.

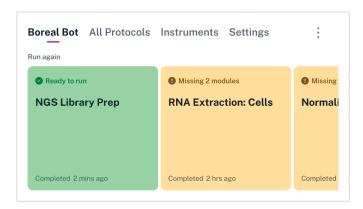
Touchscreen Operation

Use the touchscreen to control the AAW $^{\text{TM}}$ Workstation whenever the robot is on. If the touchscreen has gone to sleep, tap it once to wake the screen. If it does not wake, check if the AAW $^{\text{TM}}$ Workstation is powered on.

Robot Dashboard

The dashboard is the main screen for the robot, accessible by tapping the robot's name in the top left corner of the touchscreen.

The dashboard provides quick access to recently run protocols. It displays protocols as large cards in a horizontal carousel. Green cards show protocols that are ready to run. Orange cards show protocols that require hardware setup or have a deck configuration conflict. The dashboard can display up to eight previously run protocols.



From the dashboard you can also perform actions that apply to the robot as a whole, rather than a particular protocol. Access these actions by tapping the three-dot (:) menu:

- Home gantry: Move the gantry to its home position at the back right of the working area.
- Restart robot: Perform a soft restart of the robot.
- Deck configuration: Manage deck fixture locations.
- Lights on/off: Toggle the LED lights that illuminate the working area.

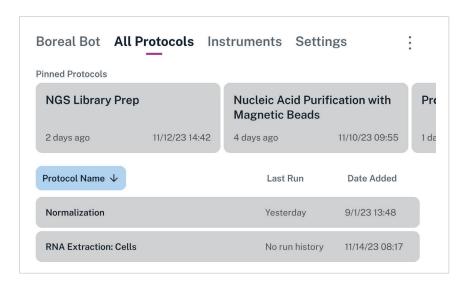
The top navigation on the dashboard provides access to the other main screens: All Protocols, Instruments, and Settings.

Protocol Management

The All Protocols screen is an interactive list of all protocols stored on the AAW™ Workstation. (Sending a protocol to AAW™ requires the Opentrons® App. See <u>Transferring Protocols to the AAW™ Workstation on page 86.</u>)

There are two sections of the All Protocols screen:

- Pinned protocols: Large cards in a horizontal carousel at the top of the screen.
- Other protocols: A vertical list at the bottom of the screen.



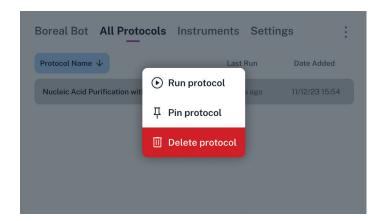
Regardless of which section a protocol is in, its card or list entry includes information about when it was last run and when it was added to this robot.

Note: The AAW[™] Workstation can store a maximum of 20 unique protocols. It automatically deletes older protocols to maintain this limit. Use the Opentrons® App if you need to manage a larger number of protocols.

Pin a Protocol

Long press on a protocol and tap Pin protocol to move it to the pinned protocols section. Conversely, long press a pinned protocol and tap Unpin protocol to remove it from the section.

You can pin up to eight protocols. When you hit the maximum, you'll need to unpin a protocol before pinning another one.



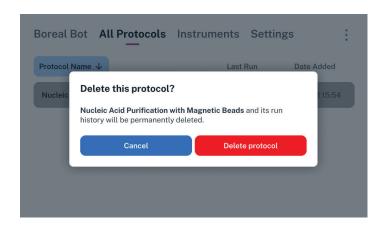
Sort Protocols

Tap any of the three headers — Protocol Name, Last Run, or Date Added — to sort the All Protocols section. Tap once to sort protocols in ascending order (A to Z for names, oldest to newest for dates). Tap again to reverse the sort order. The current sort criterion is highlighted in blue and the current sort order is indicated by an upward or downward arrow.

Delete a Protocol

Long press on a protocol and tap **Delete Protocol** to delete it directly from the All Protocols screen. The AAW™ Workstation will prompt you for confirmation that you want to delete the protocol file and all of its run history.

Warning: Run history is not recoverable after you delete a protocol on the AAW $^{\text{TM}}$ Workstation. The protocol file itself is also not recoverable, although you may be able to resend the protocol to the AAW $^{\text{TM}}$ Workstation if you've kept a copy of it on a computer.



Protocol Details

Tap on any protocol to view the Protocol Detail screen. This screen displays all of the types of information included in the protocol file, as well as common protocol actions. An indicator at the top left of the screen shows whether the protocol is ready to run, or whether you need to perform additional setup.

Action Buttons

On any of the protocol detail tabs, three action buttons are available:

- Start setup (top right)
- Pin protocol (bottom left)
- Delete protocol (bottom right)

Summary Tab

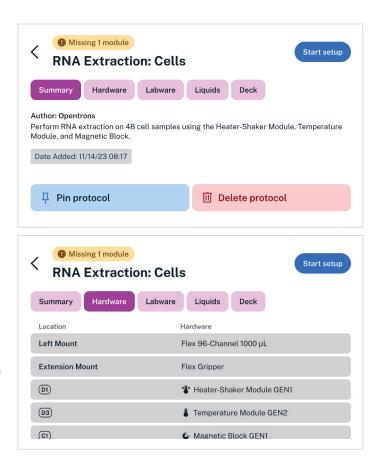
The Summary tab shows:

- Protocol name: For protocols with very long names, tap to toggle between the full and truncated name.
- Author: Who created the protocol.
- Description: For protocols with long descriptions, scroll to read the full text.
- Date added: Timestamp when the protocol file was received.

Hardware Tab

The Hardware tab is a list of all instruments, modules, and fixtures used in the protocol.

- The Location column tells you where the hardware needs to be attached to the AAW™ Workstation.
- For instruments, location can be the left pipette mount, right pipette mount, both mounts (for the 96-channel pipette), or the extension mount (for the gripper).
- For modules and fixtures, the location is the deck slot or slots that the item occupies.



Labware Tab

The Labware tab is a list of all labware used in the protocol. It shows the names and quantities of labware. It does not show their locations, since labware can be moved, added, or removed from the deck during the course of a protocol. Use the Deck tab to see initial positions of labware.

Labware verified in the AAW™ Workstation is indicated with a blue checkmark.

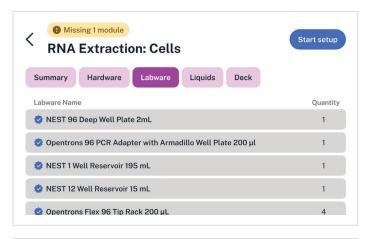
Liquids Tab

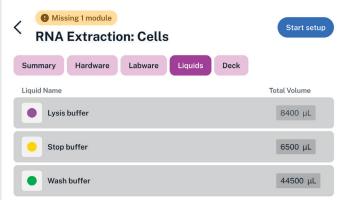
The Liquids tab lists all liquids to be loaded into labware at the start of the protocol. It shows the color code of the liquid (as assigned by the protocol author), the liquid name, and the total volume of liquid used across all wells. Use the Deck tab to see well-by-well initial positions of liquids.

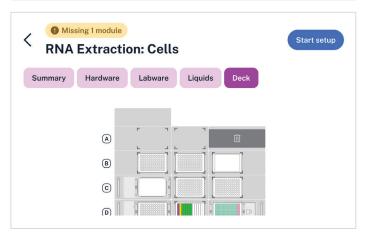
Deck Tab

The Deck tab shows a visual map of the deck at the beginning of the protocol.

For an interactive view that provides more information about the contents of each deck slot, tap Start setup, then tap Labware, and then tap Map View. Tap on any labware to see its type and custom label (if set by the protocol).





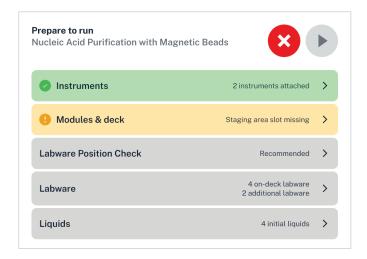


Run Setup

When you start setup for a protocol, you'll see the **Prepare to run** screen, which summarizes all of the requirements for the protocol.

If hardware is not connected or calibrated, you will see a warning icon (exclamation point) and the row will be highlighted in orange. If all requirements are met, you will see a checkmark and the row will be highlighted in green. The rows for labware and liquids are always grey, since you have to verify their positions manually before starting the run.

Tap any row with a right arrow to show more information for that category. (The one exception is tapping Labware Position Check, which begins that process. See Labware Position Check on page 77.)



Category	Description	
Instruments	See if all instruments are attached to the correct mounts and calibrated. Tap Attach or Calibrate to set up any that aren't.	
Modules	See the locations and connection statuses of hardware on the deck.	
and deck	 Tap i Setup Instructions to get detailed module instructions from the Help Center. 	
	 Tap Map View to switch to a visual layout of hardware positions. 	
Labware	See the locations of labware. Each labware lists its initial deck location, and icons indicate labware that are on top of modules. Tap Map View to switch to a visual layout of labware positions.	
Liquids	See the types and total volumes of liquids. Tap any liquid name to expand a list of well-by-well volumes. In turn, tap an individual volume row to show a visual layout of its location within labware.	

On any category screen, return to the "Prepare to run" screen by tapping the back arrow in the top left.

Labware Position Check

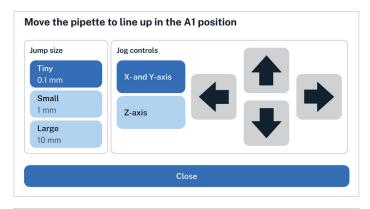
Perform the Labware Position Check before the protocol run. This process fine-tunes the positioning of instruments, relative to specific types of labware in specific slots on the deck. The results of Labware Position Check are saved as labware offsets, which are measured to the nearest 0.1 mm. Apply saved labware offsets to future runs of the same protocol (or other protocols that use the same labware in the same positions) to save time.

Labware Position Check includes these steps:

- 1. Attach the calibration probe to the pipette.
- 2. Clear the deck of labware, but leave modules in place.
- 3. Place a specific type of labware in a specific deck slot.
- 4. Align the probe to the labware using the on-screen jog controls. Then confirm the position.
- 5. Repeat steps 3 and 4 for each labware–slot combination used in the protocol.
- 6. Review and save your new labware offset data.
- 7. Remove the calibration probe from the pipette.

Jog controls used in Labware Position Check. Use larger jump sizes to move the pipette quickly, but beware of crashing the pipette into labware.

Summary of new labware offsets ready to be applied to a protocol.





When Labware Position Check is run for the first time, the pipette will start at its default position for all labware (X 0.0 Y 0.0 Z 0.0). On subsequent runs, the pipette will start at the previously saved offset locations. This provides quick confirm offset data before every protocol run.

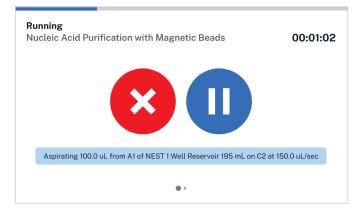
Note: The pipette will always start at the default position if Apply Labware Offsets is turned off in the robot settings.

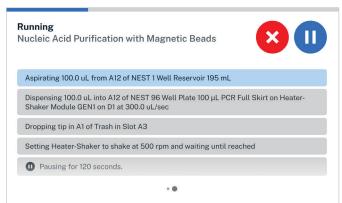
Run Progress

Once everything is set up, begin the run by tapping the play button □ on the "Prepare to run" screen. AAW[™] Workstation will begin the protocol and the Running screen will be onscreen.

The Running screen gives quick access to stop and play/pause controls. On the default view, these controls are large and only the current step of the protocol is shown.

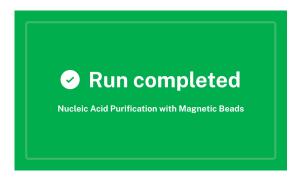
Swipe from right to left to see an alternative view with smaller controls and more protocol steps. The current step will always be at the top of the list.





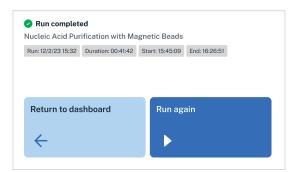
Run Completion

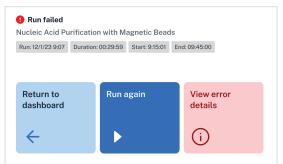
At the end of the protocol, a large "Run completed" or "Run failed" message will take over the touchscreen. These color-coded messages match the LED status bar at the top of the robot and are visible at a distance.





Tap anywhere on either of these screens to go to the run summary screen, which shows information about the protocol run time and next steps. The summary screen always gives the options to **Return to dashboard** or have the protocol **Run again**. If the run failed, **View error details** and begin the troubleshooting process.





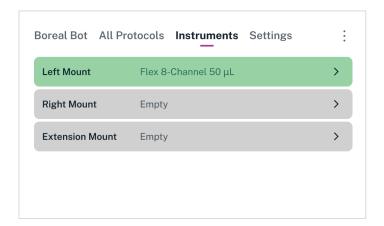
Instrument Management

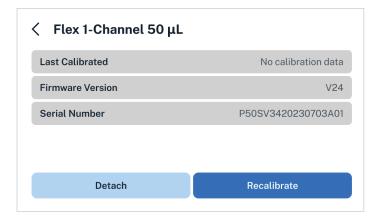
The Instruments screen is an interactive list of all instruments that you've connected to the AAW™ Workstation. The list is organized by mount: left pipette mount, right pipette mount, and extension mount.

For an empty mount, tap anywhere on the row to begin the process of attaching an instrument.

For an occupied mount, the row lists its current contents. Tap anywhere on the row to get more details about the instrument, detach it, or recalibrate it.

- Last Calibrated: The date and time of the instrument's most recent calibration.
- Firmware Version: The version of the firmware running on the instrument. The firmware is automatically updated whenever the instrument is attached, depending on the robot system version.
- Serial Number: A unique identifier for the instrument. The serial number will be required to communicate with Tech Support.





Attach an Instrument

From an empty mount, choose the type of instrument to install. Connect and secure the instrument using its captive mounting screws. For more details, follow the instructions on the touchscreen or see <u>Instrument Installation and Calibration on page 17</u>.

Installation steps depend on the instrument and the current setup of the robot. For example, if the 8-channel pipette is already attached and you attempt to install the 96-channel pipette on the other mount, the touchscreen will give instructions for detaching the 8-channel so the 96-channel can occupy both mounts.

Detach an Instrument

Choose an attached instrument to detach. Loosen the instrument's captive mounting screws and remove it from the gantry. For more details, follow the instructions on the touchscreen. Exact removal steps depend on the instrument you choose and the current setup of the robot.

Recalibrate an Instrument

Choose an attached instrument to recalibrate. Then connect the instrument's calibration probe or pin and begin the automated calibration process. For more details, follow the instructions on the touchscreen or see Instrument Installation and Calibration on page 17.

Note: The new calibration data will overwrite any previous calibration data for that instrument.

Robot Settings

The **Settings** screen lists all the behaviors that can be customized on the AAW^{TM} Workstation. On the touchscreen, scroll the list to see all the settings. Although they are presented in a single list, they roughly break down into four categories.

Setup

All of these settings are established at setup, and they can be changed at any time.

Network Settings

View the status of or set up a wireless network, Ethernet, or USB connection. Multiple connections can be active simultaneously.

Robot Name

Change the name of the robot that appears on the touchscreen dashboard and in the Opentrons® App.

Robot System Version

See the current version of the robot software or check for updates. If the AAW™ Workstation has already automatically checked for updates and found one, this item will have an "Update available" badge in the settings list.

Display

Control how information is displayed to meet the needs of the lab and users.

Status Light

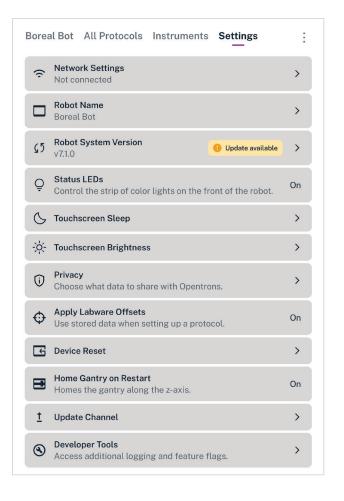
Turn on or off the strip of color lights on the front of the robot.

Touchscreen Sleep

Set how long the touchscreen should remain on when idle. The default is for the display to never go to sleep. When the screen is asleep, tap it once to wake it.

Touchscreen Brightness

Set the screen's brightness to one of six levels by tapping - or +.



Privacy

Choose what data to share with the Opentrons® network. This information is always anonymized and we only use it to improve our products.

The AAW™ Workstation records what it's doing in several log files that are stored on the robot. These logs are grouped into two categories for privacy opt-in purposes:

Robot Logs

Data about robot server activities, executed API commands, and interactions with attached modules.

Display Usage

Data about how the touchscreen draws its graphics.

Opting out of automatic data sharing will still allow user to download log files and manually send them to Tech Support for troubleshooting.

Note: There are separate privacy controls in the Opentrons® App. Turning sharing on or off from the touchscreen only affects data collected and sent by the robot. The laptop or desktop computer will still automatically share data if this feature is enabled in the Opentrons® App.

Advanced

These settings will likely only be used for troubleshooting or testing pre-release features.

Apply Labware Offsets

Choose whether to use saved offset data from Labware Position Check in subsequent protocol runs. This setting is on by default. It is recommended to run the Labware Position Check before every run. Applying previous labware offsets at the beginning of Labware Position Check can make the process quicker.

Device Reset

Batch delete certain types of information from the robot, such as calibrations, run history, or protocols.

Home Gantry on Restart

By default, the gantry moves to its home position any time the AAW™ Workstation is turned on. Only disable this behavior if there is a reason the gantry must remain stationary after powering on.

Update Channel

Choose whether to receive stable or beta software updates.

Developer Tools

Enable additional tools and features designed for developers. This is not recommended unless instructed by Technical Support.

Deck Configuration

Deck Configuration establishes which fixtures are attached to the deck and in what locations. These fixtures do not contain electronic or mechanical components and the robot will not detect them automatically. Configure deck fixtures via the touchscreen or Opentrons® App.

Mapping fixtures to deck slots allows the robot to find discrepancies between the hardware used in a protocol and what it thinks is attached to the deck. The AAW™ Workstation detects potential conflicts between the hardware setup of a protocol and the robot's current deck configuration (see <u>Resolving Deck Conflicts on page 83</u>). Running protocols with proper deck configuration helps avoid collisions among the various components installed on the robot.

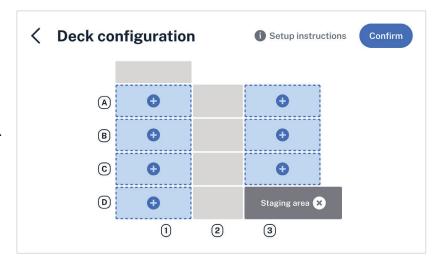
For more information on which fixtures to configure in which slots, see <u>Deck Fixtures on page 22</u>.

Adding and Removing Fixtures

To add deck fixtures via the touchscreen:

- 1. Tap the three-dot (:) menu and then tap Deck configuration. This opens the interactive deck map.
- 2. Tap a blue deck slot that you want to configure. This opens the fixture menu.
- 3. From the fixture menu, select the item you want to add.
- 4. Tap a fixture to add it to the deck.
- 5. Tap Confirm.

Click the X on a fixture on the deck map to remove it from the deck configuration.



The touchscreen image shows the deck configured with a staging area slot in D3, and no other fixtures. The deck can also be configured in the Opentrons® App, on the robot details page.

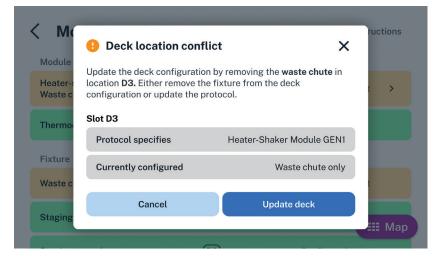
Resolving Deck Conflicts

The AAW™ Workstation displays orange warning prompts when setting up a protocol run that conflicts with the current deck configuration. To resolve the conflict:

- Tap the prompt for more information on what the protocol specifies, compared to the current deck configuration.
- Inspect the hardware configuration and attach, move, or remove the deck fixtures or modules as needed.
- 3. Tap Update deck to clear the conflict warning.

Alternatively, you can modify your protocol to fit your current deck configuration, and then resend it to your AAW™ Workstation.

The AAW™ Workstation will not run a protocol until you resolve all deck conflict warnings.



The touchscreen image above shows the error message that occurs when a protocol requires a Heater-Shaker in slot D3, but the deck configuration indicates that the waste chute is in that location.

Fixture Statuses

The following table defines the statuses the robot generates when it compares its configured deck fixtures to the protocol.

Status	Description A fixture is specified in the correct location. Always verify that the fixture is physically attached before running the protocol.	
Configured		
Location conflict	A deck slot is configured with a fixture different from the fixture specified in your protocol (e.g., the protocol specifies a waste chute, but deck slot D3 is occupied by a staging area slot).	
Not configured	A fixture required by your protocol is missing from the deck configuration (e.g., the protocol requires a staging area slot but that fixture is not configured in the specified location).	

Configuration Statuses

The following table defines the deck configuration statuses the robot generates when it compares the attached instruments and attached modules to the deck configuration and the protocol.

Status	Description	
Attach pipette	A required pipette is not attached.	
Calibrate	A module needs calibration. It is in the right location and connected to the robot.	
Calibrate pipette	An attached pipette requires calibration.	
Connected	Modules are connected, calibrated, and in the right locations. Configuration status is good.	
Location conflict	A module location conflicts with a deck fixture.	
Not connected	The module is not connected to the robot or is powered off. Once connected, there will be no location conflict.	

The Opentrons® App

App Installation

Download the Opentrons® App at https://opentrons.com/ot-app/. The app requires Windows 10, macOS 10.10, or Ubuntu 12.04 or later. The app may run on other Linux distributions, but they are not officially supported.

Windows

The Windows version of the Opentrons® App is packaged as an installer. To use it:

- 1. Open the .exe file you downloaded from Opentrons.com.
- 2. Follow the instructions in the installer. You can install the app for a single user or all users of the computer.

The app opens automatically once installed. Grant the app security or firewall permissions, if prompted, to make sure it can launch and communicate with the AAW™ Workstation over your network.

macOS

The macOS version of the Opentrons® App is packaged as a disk image. To use it:

- 1. Open the .dmg file you downloaded from <u>Opentrons.com</u>. A window for the disk image will open in Finder.
- 2. Drag the Flex icon onto the Applications icon in the window.
- 3. Double-click on the Applications icon.
- 4. Double-click on the Flex icon in the Applications folder.

Grant the app security or firewall permissions, if prompted, to make sure it can launch and communicate with the AAW™ Worksation over your network.

Ubuntu

The Ubuntu version of the Opentrons® App is packaged as an AppImage. To use it:

- 1. Move the .AppImage file you downloaded from <a>Opentrons.com to your Desktop or Applications folder.
- 2. Right-click the .AppImage file and choose Properties.
- 3. Click the Permissions tab. Then check Allow executing file as a program. Close the Properties window.
- 4. Double-click the .AppImage file.

Note: Do not use third-party AppImage launchers with the Opentrons® App. They may interfere with app updates. Using third-party launchers to control Opentrons® products or AAW^{TM} Workstations is not supported.

Transferring Protocols to the AAW™ Workstation

Every protocol will begin as a file on the computer, regardless of what method of Protocol Development used. The protocol must be imported into the Opentrons® App and then transfer it to your AAW $^{\text{\tiny TM}}$ Workstation. When transferring a protocol, you can choose to begin run setup immediately or postpone as needed.

Import a Protocol

The Opentrons® App opens to the Protocols screen. (Click **Protocols** in the left sidebar to return to this screen.)

- 1. Click **Import** in the top right corner to reveal the Import a Protocol pane.
- 2. Click **Choose File** and find your protocol in the system file picker, or drag and drop your protocol file into the well.

The Opentrons® App will analyze your protocol as soon as you import it. Protocol analysis is the process of taking the JSON object or Python code contained in the protocol file and turning it into a series of commands that the robot can execute in order. If there are any errors in your protocol file, or if you're missing custom labware definitions, a warning banner will appear on the protocol's card. Correct the errors and re-import the protocol. If there are no errors, your protocol is ready to transfer to the AAW™ Workstation. See Actions available in the three-dot menu (:), for imported protocols (below).

Note: In-app protocol analysis is only a preliminary check of the validity of your protocol. Protocol analysis will run again on the robot once the protocol is transferred to it. It's possible for analysis to fail in the app and succeed on the robot, or vice versa. Analysis mismatches may occur when your app and robot software versions are out of sync, or if you have customized the Python environment on your Flex.



Run Immediately

Click the three-dot menu (\vdots) on your protocol and choose **Start setup**. Choose a connected and available AAWTM Workstation from the list to transfer the protocol and begin run setup immediately. The run setup screen will appear both in the app and on the touchscreen, and you can continue from either place.

If you stay in the app, expand the sections under the Setup tab and follow the instructions in each one: Robot Calibration, Module Setup (if your protocol uses modules), Labware Position Check (recommended), and Labware Setup. Then click Start run to to begin the protocol.

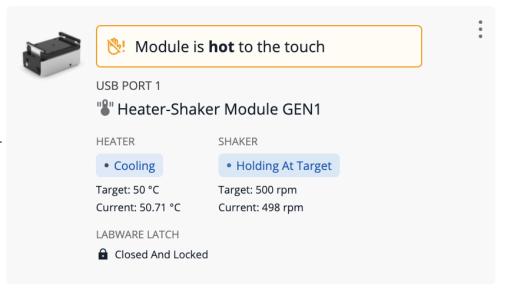
If you move to the touchscreen, follow the steps in Run Setup on page 76.

Run Later

Click the three-dot menu (i) on your protocol and choose **Send to AAW™ Workstation**. Choose a connected and available AAW™ Workstation from the list to transfer the protocol. A message indicating a successful transfer will pop up both in the app and on the touchscreen. To set up your protocol, you need to move to the touchscreen and follow the steps in <u>Run Setup on page 76</u>.

Module Status and Controls

Use the Opentrons® App to view the status of modules connected to your AAW™
Workstation and control them outside of protocols. Click
Devices and then click on your AAW™ Workstation to view its robot details page. Under Instruments and Modules, there is a card for each attached module. The card shows the type of module, what USB port it is connected to, and its current status.



Note: The Magnetic Block does not have a card in Instruments and Modules, since it is unpowered and does not connect to the robot via USB.

Click the three-dot menu (:) on the module card to choose from available commands. The **About** module will also show the firmware version and serial number of the module (This information is very useful when contacting Tech Support). The other commands depend on the type of the module and its current status:

Module type	Commands	
Heater-Shaker	Set module temperature/Deactivate heater Open labware latch/Close labware latch Test shake/Deactivate shaker	
Temperature	Set module temperature/Deactivate module	
Thermocycler	Set lid temperature/Deactivate lid Open lid/Close lid Set block temperature/Deactivate block	

Recent Protocol Runs

From the touchscreen, the robot details page lists a maximum of 20 recent protocol runs. This provides additional information compared to the touchscreen, which only shows the most recent run for each unique protocol.

Each entry in the recent protocol runs list includes the protocol name, its timestamp, whether the run was canceled or completed, and the duration of the run. Click the disclosure triangle next to any run to show its associated labware offset data. Click the three-dot menu (:) for related actions:

View protocol run record

Show the protocol run screen as it appeared when the protocol ended (succeeded, failed, or was canceled), including all performed steps.

Rerun protocol now

The same as choosing Start setup on the corresponding protocol.

Download run log

Save to your computer a JSON file containing information about the protocol run, including all performed steps.

Delete protocol run record

Delete all information about this protocol run from AAW™, including labware offset data. This option will remove evidence the protocol run happened.

Note: To maintain a comprehensive record of all runs performed on your AAW[™] Workstation, the Download Run Log feature must be used to save this information to your computer. The AAW[™] Workstation will not retain information about more than 20 runs on the robot. Proceeding to the Run Setup screen generates an entry in the list and counts towards the maximum of 20 runs, even if you never begin the protocol.

Advanced Operation

Jupyter Notebook

The Opentrons® <u>Jupyter Notebook</u> server is available on port 48888. Use the Jupyter Notebook to individually run discrete chunks of Python code, called cells. This is a convenient environment for writing and debugging protocols, since different parts of the protocol can be defined in different notebook cells, and run a single cell at a time.

Access the robot's Jupyter Notebook:

- In the Opentrons® App. Go to Devices > your robot > Robot Settings > Advanced and then click Launch Jupyter Notebook.
- In your web browser. Navigate directly to http://<robot-ip>:48888, replacing <robot-ip> with the local IP address of your AAW™ Workstation.

Additional information is available at https://jupyter-server.readthedocs.io/en/latest/.

Command-Line Opration over Secure Shell (SSH)

Connect over Secure Shell (SSH) to run protocols directly from the command line or to perform advanced tasks, such as customizing the Python environment on the robot. Protocols that reference external files on disk (apart from custom labware definition files) must be run from the command line.

Set up SSH access before attempting to connect. Setup requires a bash or zsh shell with openssh installed. In your shell, generate private and public key files by running:

```
ssh-keygen -f robot_key
```

Enter a passphrase when prompted. It is not recommended to leave this empty.

Next, use a USB-A flash drive to copy the public key file to the robot. Connect the drive to the computer and copy robot_key.pub to the root of the drive. Then eject the drive and connect it to an open USB-A port on your robot.

Note: The flash drive must be formatted with a standard filesystem readable by the embedded Linux system on the AAW™ Workstation. HFS- and APFS-formatted drives are not supported.

On your computer, post an HTTP request to read the public key file from the drive. Replace ROBOT_IP with the local IP address of your AAW $^{\text{TM}}$ Workstation:

```
curl \
--location --request POST \
'http://ROBOT_IP:31950/server/ssh_keys/from_local'
```

The transfer is successful if you get a 201 response with a message indicating how many keys were added. If the transfer fails, you will get a 404 response.

Once the transfer is complete, connect to your robot, again replacing ROBOT_IP with the local IP address of your AAW™ Workstation:

```
ssh -i robot_key root@ROBOT_IP
```

The connection was sucessful when an ASCII art version of the logo appears.

Maintenance and Service

Users should perform routine maintenance and cleaning tasks. If problems occur, service and repairs can be schedued at SigmaAldrich.com/TechService.

Cleaning your AAW™ Workstation

Routine cleaning helps keep the AAW^{TM} Workstation free of contaminants that can affect your protocols. Cleaning is an opportunity to inspect the AAW^{TM} Workstation, pipettes, gripper, modules, and other hardware for wear or damage.

If you have any questions about cleaning the AAW™ Workstation and its related components, contact SigmaAldrich.com/TechService.

Before You Begin

The AAW™ Workstation is an electrically powered mechanical device. Unplug the robot before cleaning. Remove any instruments, modules, and labware before cleaning the robot. Removing attached items gives you more room to work and provides better access to the deck, gantry, and other spaces.

What You Can Clean

Wipe off all the visible and easily accessible surfaces of the AAW™ Workstation. This includes the exterior and interior frame, touchscreen, windows, gantry, and deck. The AAW™ Workstation does not have any internal parts to open or disassemble for this level of maintenance.

Cleaning Solutions

The following table lists the chemicals you can use to clean the AAW™ Workstation. Diluted alcohol and distilled water are our recommended cleaning solutions, but you can refer to this list for other cleaning options. You can also use these chemicals to clean modules, pipettes, and other attached hardware.

Warning: Do not use acetone. The robot, pipettes, and modules can be damaged or dissolve with acetone.

Solution	Recommendations	
Alcohol	Includes ethyl/ethanol, isopropyl, and methanol. Dilute to 70% for cleaning. Do not use 100% alcohol.	
Bleach	Dilute to 10% (1:10 bleach/water ratio) for cleaning. Do not use 100% bleach.	
Distilled water	You can use distilled water to clean or rinse your robot.	

Frame and Window Panel Cleaning

- 1. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 2. Gently wipe off the exposed and easily accessible surface areas.
- 3. Use a cloth dampened with distilled water for a rinse wipedown.
- 4. Let the robot air dry.

Deck Cleaning

- 1. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 2. Gently wipe off the deck, deck slots, and trash bin. Remove the deck slots and trash bin for easier access.
- 3. Use a cloth dampened with distilled water for a rinse wipedown.
- 4. Let the deck pieces air dry. Replace any pieces removed for cleaning.

Gantry Cleaning

- 1. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 2. Gently wipe off the horizontal and vertical gantry surfaces, and side rails.
- 3. Use a cloth dampened with distilled water for a rinse wipedown.
- 4. Let the gantry air dry.

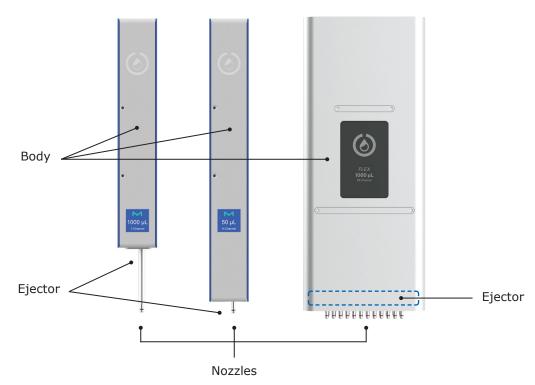
Waste Chute Cleaning

- 1. Remove the waste chute from its deck plate adapter.
- 2. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 3. Gently wipe down the exterior of the chute. The interior is powder-coated steel, so it can tollerate mild detergents or surfactants.
- 4. Use a cloth dampened with distilled water for a rinse wipedown.
- 5. Let the waste chute air dry and reattach it to the deck.

Cleaning Pipettes and Tips

To clean a 1-, 8-, or 96-channel pipette:

- 1. Remove the pipette from the gantry.
- 2. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 3. Gently wipe down the following parts:
 - Body
 - Ejector
 - Nozzles
- 4. Use a cloth dampened with distilled water for a rinse wipedown.
- 5. Let the pipette air dry and reattach.



Pipette Decontamination

The routine cleaning steps described previously may not clean the pipette if it becomes contaminated with substances like nucleic acids, proteins, or radioactive material. When a pipette becomes contaminated, perform the decontamination steps below. Contact SigmaAldrich.com/TechService if the pipette is contaminated and these cleaning procedures do not work.

Outside of the Pipette

Refer to the following table for recommended cleaning methods, by contamination type.

Contaminant	Cleaning recommendation	
Aqueous solutions	Rinse the contaminated parts with distilled water or 70% ethanol and air dry at 15.5 °C (60 °F).	
Nucleic acids	Clean the contaminated parts in a glycine/HCl buffer (pH 2) for 10 minutes, rinse with distilled water, and air dry.	
Organic solvents	Allow the solvent to evaporate on its own or immerse the pipette nozzle only in a detergent, rinse with distilled water, and air dry.	
Proteins	Clean the contaminated parts with a detergent, rinse with distilled water, and air dry. Do not use alcohol. That will set the proteins.	
Radioactive materials	Place the pipette nozzle in a solution like Decon 90, rinse with distilled water, and air dry.	

Inside the Pipette

Filtered pipette tips help prevent contaminating the barrel or inside of the pipette. But, you cannot disassemble the barrel if it becomes contaminated. If the inside of the pipette gets contaminated, the following steps may help remove the contamination:

- 1. Inject a small amount of cleaning solution into the barrel using a manual pipette or syringe.
- 2. Gently shake the pipette to swirl the cleaning solution.
- 3. Rinse with distilled water.
- 4. Let the pipette air dry and reattach.

Cleaning Pipette Tips

Pipette tips are disposable items. While attached tips can be reused if the protocol allows it, they are not designed to be removed, cleaned, and then reattached. Also, cleaning pipette tips in a parts washer or an autoclave can cause them to warp. Discard pipette tips after use. See <u>Product Ordering on page 103</u>.

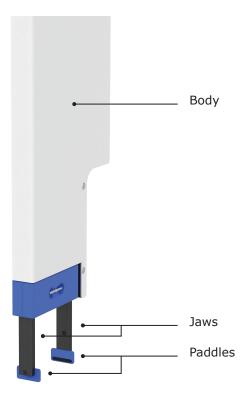
Cleaning the AAW™ Flex Gripper

To clean the gripper:

- 1. Remove the gripper from the gantry.
- 2. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 3. Gently wipe down the following parts:
 - · Gripper body
 - Jaws
 - Paddles
- 4. Use a cloth dampened with distilled water for a rinse wipedown.
- 5. Let the gripper air dry and reattach.

Warning:

- Do not disassemble the AAW™ Flex Gripper for cleaning or attempt to clean its internal electronic components.
- Do not put the gripper in an autoclave. The high temperatures, pressures, and steam used inside an autoclave can damage the electronics, circuit boards, small electric motors, and other sensitive components.



Gripper Paddles

The gripper paddles are wear items that require periodic replacement. When cleaning the AAW[™] Flex Gripper, inspect the rubber pads for tears, nicks, or other wear. Replace the paddles as needed with the two spares (included with the AAW[™] Flex Gripper). For additional gripper pads, see Product Ordering on page 103.

Note: Aggressive cleaning chemicals may reduce the lifetime of the rubber pads on the gripper paddles.

Cleaning Modules

The surfaces of any modules should be cleaned. The general procedure is the same for all supported modules: Heater-Shaker, Magnetic Block, Temperature, and Thermocycler.

Be sure to power off and unplug modules before cleaning. You can clean the top surfaces of modules while they're installed in a deck slot. However, for better access:

- Remove the caddy and module from the deck slot.
- Remove the module from the caddy.
- Disconnect any USB or power cables (if you're cleaning a powered module).

Warning:

- Do not disassemble modules for cleaning or attempt to clean their internal electronic components.
- Do not put modules in an autoclave. The high temperatures, pressures, and steam used inside an autoclave can damage the electronics, circuit boards, small electric motors, and other sensitive components.

General Module Cleaning

- 1. Dampen a soft, clean cloth or paper towel with a cleaning solution.
- 2. Gently wipe off the module's surfaces.
- 3. Use a cloth dampened with distilled water as a rinse wipedown.

4. Let the module air dry.

Thermocycler Seals

To set up the Thermocycler with a clean seal:

- 1. Affix a seal to the Thermocycler lid (if one is not attached already).
- 2. Wipe the seal with a 1:10 diluted bleach solution.
- 3. Rinse the seal with molecular biology grade water.
- 4. Let the seal air dry.



Autoclave-Safe Labware

Please visit the labware manufacturer's website before autoclaving any item. In general, plastic labware is designed to be consumable. If you aren't sure whether a piece of labware is autoclave-safe, it's best to discard it and replace it with new, clean labware. The following list is considered suitable for autoclave.

Labware Type	Autoclave-Safe Items	
Reservoirs	All NEST™ reservoirs	
Well plates	Thermo Scientific® Nunc 96-well Plate, 1300 μL Thermo Scientific® Nunc 96-well Plate, 2000 μL USA Scientific® 96-well Deep Well Plate, 2.4 mL	
Sample vials	Eppendorf TM Safe-Lock 1.5 mL and 2.0 mL vials (when left open at 121 $^{\circ}$ C, 20 min)	

Servicing the AAW™ Workstation

The AAW™ Workstation is designed for years of full-time operation. Repairs or service should only be done by personel authorized from <u>SigmaAldrich.com/TechService</u>. There are multiple levels of service. See <u>Product Ordering on page 103</u> or contact <u>SigmaAldrich.com/TechService</u> for assistance.

Additional Services

We offer two levels of service, Flex Care and Flex Care Plus, both of which include benefits for onboarding, maintenance, repair, and more. Both services include:

- Onboarding with a field applications scientist.
- Parts for yearly preventive maintenance.
- Access to a dedicated support engineer.
- Extension of the warranty

In addition, Flex Care Plus includes on-site visits for:

- Installation.
- Preventive maintenance, yearly.
- Repairs, as needed.

Services can also be ordered a la carte, including installation, protocol development (remote or on-site), repair, relocation, and preventive maintenance. Contact SigmaAldrich.com/TechService for more information.

Installation Qualification and Operation Qualification

The Installation Qualification and Operation Qualification (IQ/OQ) will be performed after the first set up. This should also be run after hardware is attached, and after service has been performed.

IQ/OQ verifies:

- The serial numbers of your hardware, including the AAW™ Workstation, pipettes, gripper, and modules.
- Software and firmware versions.
- Proper connection of attached hardware.
- Pipette and module calibrations.

Consult the SigmaAldrich.com/TechService for a complete checklist of IQ/OQ activities to perform.

Preventative Maintenance

In addition to routine cleaning, certain simple maintenance tasks should be performed on a regular schedule. The timetable below are based on 20 hours of operation per week, 50 weeks per year. Adjust the schedule more or less as needed to meet lab requirements.

Frequency	Task	Description
Daily	Empty trash	Take the movable trash out of its deck slot, safely discard its contents, and replace it into the deck.
	Inspect working area	Clear the deck of any debris, liquid, or no-longer-needed labware. Check that the plastic labware clips are not damaged.
Weekly	Clean surfaces	Clean the exterior surfaces of the deck, gantry, windows, instruments, and other hardware, as described above.
Monthly	Power cycle	Turn off the AAW $^{\mbox{\tiny TM}}$ Workstation and all connected modules. Then turn them back on.
Every six months	Inspect pipettes	Inspect the O-rings on the pipette nozzles for signs of wear (notches, deformation). Replace the O-rings with the provided spares, if needed.
	Inspect gripper	Inspect the rubber pads on the gripper paddles. Replace the paddles with the provided spares, if needed.
	Recalibrate instruments	Run recalibration for the pipettes and gripper.
Yearly	Evaluate pipette performance	Evaluate the overall performance of the pipettes. Replace any pipette that is not performing according to the <u>Pipette Specifications on page 27</u> .

The annual on-site preventative maintenance visit that is part of Flex Care Plus includes performance of the tasks listed above, as well as replacement parts.

Additional Documentation

SigmaAldrich.com

The AAW™ Workstation product page on <u>SigmaAldrich.com</u> provides the most current downloadable PDFs of this user guide, quick start guide, safety sheet and other documents.

Opentrons® Knowledge Hub

The Opentrons® Knowledge Hub hosts publications about products and related scientific applications. Some of the publication categories are listed below. Additional information is also available at SigmaAldrich.com.

Application Notes

Scientific papers on performing particular applications using the AAW $^{\text{\tiny{TM}}}$ Workstation and Opentrons Flex $^{\text{\tiny{TM}}}$ robot. Topics range from nucleic acid extraction and NGS quantification to handling volatile or viscous liquids.

Certificates

Official regulatory and compliance documents.

Documentation & Manuals

Product instruction manuals for robots and modules. Also includes digital versions of the Quick Start Guides that ship in product packages.

White Papers

Documents that detail how products are constructed and validated. White papers include dimensional drawings of hardware.

Python Protocol API documentation

The <u>online documentation for the Python Protocol API</u> describes how to write automated biology lab protocols for AAW^{TM} Workstation and hardware modules. The documentation includes a tutorial for users writing their first Python protocol.

The Python API documentation covers writing Python code to:

- Load and work with labware.
- · Load and work with modules.
- Load and work with pipettes and the gripper.
- Perform discrete liquid-handling actions, such as aspirating and dispensing.
- Perform complex liquid-handling actions, such as transfers between wells.
- Move instruments to exact locations in the working area.

There is also a Python API reference with information about all of the classes and methods that comprise the API.

Opentrons® HTTP API Reference

The <u>HTTP API reference</u> describes all of the endpoints of the API used to directly control the robots. The API has many endpoint categories, including:

- Querying the state of the robot
- Performing calibration tasks
- · Managing and running protocols
- · Moving the gantry and instruments
- Controlling discrete systems like the ambient lighting and camera

The API reference is defined by and generated from an OpenAPI specification.

Developer Documentation

Documentation for working directly with source code is available alongside the corresponding code on GitHub. Notable documentation pages include:

Development Environment Setup

Opinionated instructions for setting up your computer to work on the software in the Opentrons® repository. These setup steps are required for running the Opentrons® App or a simulated robot server from source.

Opentrons® Emulation Wiki

Explanation and instructions for using our software that emulates AAW^{TM} robots at the firmware or hardware level.

Open-Source Software

Open-source software and hardware make science better. Code is available on GitHub and contributions from the open-source community are welcomed.

Opentrons® on GitHub

The GitHub organization can be found at https://github.com/opentrons. All of our publicly hosted code resides there, including the AAW™ robot software, the Opentrons® App, and our Python and HTTP APIs.

Our GitHub site has several useful resources for users. Users can participate in the community without being a coder.

Note: As you browse our GitHub repositories, you will encounter references to OT3, which is a model identifier for Opentrons Flex[™]. If you're having trouble finding something when searching for "Flex", try searching for "ot3" or "OT-3" instead.

Releases

New versions of the robot software and Opentrons® App are published on GitHub. Past releases of the software are kept at https://github.com/opentrons/opentrons/opentrons/releases.

Running the latest software version is recommended. However, some users need access to previous versions (e.g., for validation or compliance purposes). Download previous versions of the Opentrons® App or robot software are available under the Assets section of each release entry on GitHub.

Opening Issues

Contact <u>SigmaAldrich.com/TechService</u> first, if you're having a problem with your robot. Typically there are two types of issue reports: bugs and feature requests.

Contributing Code

Contribute code by following the development environment setup instructions. Work on the code in your own fork and then create a pull request to contribute to the codebase. For additional details on creating pull requests, including testing requirements, see the contributing guide.

Open-source Licenses

Most repositories are licensed under the Apache License 2.0, but some use other licenses. Consult the license on each repository before using or modifying the code it contains. Keep in mind that any code you contribute will be governed by the license in place on the corresponding repository.

Opentrons® Monorepo

Most of the software is in the Opentrons/Opentrons Monorepo; a single repository that contains multiple software projects, each in its own directory. The README.md file in each directory describes the project and gives advice on working with the code. The default branch in the monorepo is called edge.

The following (non-exhaustive) list of directories, subdirectories, and files can help you navigate the monorepo and find relevant code.

Path	Description	
<u>/api/</u>	Source for the Python Protocol API, written in Python and distributed as the Opentrons® PyPI package.	
/api/docs/	Documentation for the Python Protocol API, written in ReStructuredText.	
/api/release-notes.md	Release notes for the robot system software (as a whole, including changes outside of the /api/ directory).	
/app-shell-odd/	Electron application wrapper for the touch screen software — "odd" stands for ondevice display.	
/app-shell/	Electron application wrapper for the Opentrons® App.	
/app-shell/build/ release-notes.md	Release notes for the Opentrons® App (as a whole, including changes outside of the /app-shell/ directory).	
/app/	Source for the Opentrons® App. Use make commands in this directory to run the app from source.	
/labware-library/	Source for the Labware Library website.	
/protocol-designer/	Source for Protocol Designer, our no-code web application for creating JSON protocol files.	
/robot-server/	The web service that runs the Opentrons® HTTP API. The Opentrons® App and touchscreen use HTTP API calls to control the robot. You can also write your own software that makes HTTP API calls or use software like curl or Postman to make individual calls to a robot.	
/shared-data/	Special directory for data that needs to be shared between projects.	
/shared-data/labware/	Schema and labware definitions for verified labware. The Python Protocol API and Labware Library both use the definition files stored here.	
/shared-data/python/	Source for the opentrons-shared-data Python package, which is a dependency of the main package.	

Other Repositories

Software is also maintained outside of the monorepo. A few key repositories include:

Repository	Description	
buildroot	The Opentrons® fork of Buildroot, the embedded Linux system builder, which is used to configure and build the robots' operating systems.	
<u>oe-core</u>	The heart of openembedded system definitions.	
opentrons-emulation	Emulation of robots and modules at either the hardware or firmware level. Uses Docker, configuration files, and a simple command-line interface.	
opentrons-modules	Firmware for all modules.	
ot3-firmware	Firmware and peripheral systems.	
<u>protocols</u>	All protocols are hosted publicly on the Opentrons® Protocol Library. To find a protocol, use the Protocol Library website rather than this repository.	

Support and Contact Information

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Support

Answers to many common questions are available on the <u>Opentrons Help Center</u>. Contact <u>SigmaAldrich.com/TechService</u> directly if you can't find an answer to your question. We can also help you with warranty issues, parts, repairs, supplies, and other support-related problems or concerns.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/Terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/Offices.

Product Ordering

Order products at SigmaAldrich.com.

AAW™ Automated Assay Workstation - Assay Ready

Order by specifying which power cord is needed for your location (right).

This product includes:			
AAW™ Automated Assay Workstation			
AAW™ 1-Channel Pipette, 1000μL			
AAW™ 8-Channel Pipette, 1000µL			
AAW™ Flex Gripper			
Opentrons Flex™ Magnetic Block			
Opentrons Flex™ Deck Expansion (4)			
Opentrons Flex™ Heater-Shaker, with 4 adapters:			
Universal Flat Adapter			
Flat Bottom Plate Adapter			
PCR Adapter			
Deep Well Adapter			

Select Power Cord For	Catalogue Number
Europe (EU)	99900256-1EA-EU
United States (US)	99900256-1EA-US
United Kingdom (UK)	99900256-1EA-UK
Italy (IT)	99900256-1EA-IT
India (IN)	99900256-1EA-IN
Israel (IL)	99900256-1EA-IL
Japan (JP)	99900256-1EA-JP
Switzerland (CH)	99900256-1EA-CH
Australia (AU)	99900256-1EA-AU
China (CN)	99900256-1EA-CN
South Africa (ZA)	99900256-1EA-ZA

Workstation and Modules

Description	Country	Catalogue Number
AAW™ Workstation	EU	99900241-1EA-EU
(Includes base only)	US	99900241-1EA-US
	UK	99900241-1EA-UK
	IT	99900241-1EA-IT
	IN	99900241-1EA-IN
	IL	99900241-1EA-IL
	JР	99900241-1EA-JP
	CH	99900241-1EA-CH
	AU	99900241-1EA-AU
	CN	99900241-1EA-CN
	ZA	99900241-1EA-ZA
Opentrons Flex [™]	EU	99900157-1EA-EU
Heater-Shaker	US	99900157-1EA-US
Module	UK	99900157-1EA-UK
	IT	99900157-1EA-IT
	IN	99900157-1EA-IN
	IL	99900157-1EA-IL
	JР	99900157-1EA-JP
	СН	99900157-1EA-CH
	AU	99900157-1EA-AU
	CN	99900157-1EA-CN
	ZA	99900157-1EA-ZA

Description	Country	Catalogue Number
Opentrons Flex [™]	EU	99900209-1EA-EU
Thermocycler	US	99900209-1EA-US
Module	UK	99900209-1EA-UK
	IT	99900209-1EA-IT
	IN	99900209-1EA-IN
	IL	99900209-1EA-IL
	JP	99900209-1EA-JP
	CH	99900209-1EA-CH
	AU	99900209-1EA-AU
	CN	99900209-1EA-CN
	ZA	99900209-1EA-ZA
Opentrons Flex [™]	EU	99900220-1EA-EU
Temperature	US	99900220-1EA-US
Module	UK	99900220-1EA-UK
	ΙΤ	99900220-1EA-IT
	IN	99900220-1EA-IN
	IL	99900220-1EA-IL
	JP	99900220-1EA-JP
	CH	99900220-1EA-CH
	AU	99900220-1EA-AU
	CN	99900220-1EA-CN
	ZA	99900220-1EA-ZA

Modules

Description	Country	Catalogue Number	Description	Country	Catalogue Number
Opentrons Flex™	EU	99100115UN-1EAEU	Opentrons Flex [™]	EU	991003503-1EA-EU
Heater-Shaker,	US	99100115UN-1EAUS	Temperature Module,	US	991003503-1EA-US
Universal Adapter	UK	99100115UN-1EAUK	Flat Bottom	UK	991003503-1EA-UK
	IT	99100115UN-1EAIT		IT	991003503-1EA-IT
	IN	99100115UN-1EAIN		IN	991003503-1EA-IN
	IL	99100115UN-1EAIL		IL	991003503-1EA-IL
	JP	99100115UN-1EAJP		JP	991003503-1EA-JP
	СН	99100115UN-1EACH		СН	991003503-1EA-CH
	AU	99100115UN-1EAAU		AU	991003503-1EA-AU
	CN	99100115UN-1EACN		CN	991003503-1EA-CN
	ZA	99100115UN-1EAZA		ZA	991003503-1EA-ZA
Opentrons Flex™	EU	99100115FL-1EA-EU	Opentrons Flex™	EU	991003500-1EA-EU
Heater-Shaker,	US	99100115FL-1EA-US	Temperature Module,	US	991003500-1EA-US
Flat Bottom Plate	UK	99100115FL-1EA-UK	1.5/2.0 mL Tube Block	UK	991003500-1EA-UK
	IT	99100115FL-1EA-IT		IT	991003500-1EA-IT
	IN	99100115FL-1EA-IN		IN	991003500-1EA-IN
	IL	99100115FL-1EA-IL		IL	991003500-1EA-IL
	JP	99100115FL-1EA-JP		JР	991003500-1EA-JP
	СН	99100115FL-1EA-CH		СН	991003500-1EA-CH
	AU	99100115FL-1EA-AU		AU	991003500-1EA-AU
	CN	99100115FL-1EA-CN		CN	991003500-1EA-CN
	ZA	99100115FL-1EA-ZA		ZA	991003500-1EA-ZA
Opentrons Flex™	EU	99100115PC-1EA-EU	Opentrons Flex™	EU	991003501-1EA-EU
Heater-Shaker,	US	99100115PC-1EA-US	Temperature Module,	US	991003501-1EA-US
PCR Plate	UK	99100115PC-1EA-UK	96-Well PCR plate	UK	991003501-1EA-UK
	IT	99100115PC-1EA-IT		IT	991003501-1EA-IT
	IN	99100115PC-1EA-IN		IN	991003501-1EA-IN
	IL	99100115PC-1EA-IL		IL	991003501-1EA-IL
	JP	99100115PC-1EA-JP		JР	991003501-1EA-JP
	СН	99100115PC-1EA-CH		СН	991003501-1EA-CH
	AU	99100115PC-1EA-AU		AU	991003501-1EA-AU
	CN	99100115PC-1EA-CN		CN	991003501-1EA-CN
	ZA	99100115PC-1EA-ZA		ZA	991003501-1EA-ZA
Opentrons Flex™	EU	99100115DE-1EA-EU	Opentrons Flex™	EU	991003502-1EA-EU
Heater-Shaker, Deep Well Plate	US	99100115DE-1EA-US	Temperature Module, 96-Well Deep Well plate	US	991003502-1EA-US
	UK	99100115DE-1EA-UK		UK	991003502-1EA-UK
	IT	99100115DE-1EA-IT		IT	991003502-1EA-IT
	IN	99100115DE-1EA-IN		IN	991003502-1EA-IN
	IL	99100115DE-1EA-IL		IL	991003502-1EA-IL
	JP	99100115DE-1EA-JP		JP	991003502-1EA-JP
	CH	99100115DE-1EA-CH		CH	991003502-1EA-CH
	AU	99100115DE-1EA-AU		AU	991003502-1EA-AU
	CN	99100115DE-1EA-CN		CN	991003502-1EA-CN
	ZA	99100115DE-1EA-ZA		ZA	991003502 1EA CN 991003502-1EA-ZA

Accessories

Description	Catalogue Number
AAW™ Workstation Installation	30200014-1EA-EU
Opentrons® Care Plus Plan	30200201-1EA-EU
Opentrons® 50 µL tip rack	99100101-20EA-EU
Opentrons® 50 μL tip rack, filtered	99100104-20EA-EU
Opentrons® 200 µL tip rack	99100102-20EA-EU
Opentrons® 200 μL tip rack, filtered	99100105-20EA-EU
Opentrons® 1000 µL tip rack	99100103-20EA-EU
Opentrons® 1000 μL tip rack, filtered	99100106-20EA-EU
Opentrons® 50 µL tip refill	99100107-20EA-EU
Opentrons® 50 µL tip refill, filtered	99100110-20EA-EU
Opentrons® 200 µL tip refill	99100108-20EA-EU
Opentrons® 200 µL tip refill, filtered	99100111-20EA-EU
Opentrons® 1000 µL tip refill	99100109-20EA-EU
Opentrons® 1000 μL tip refill, filtered	99100112-20EA-EU
Opentrons Flex™ Magnetic Block	99900204-1EA-EU
Opentrons Flex™ Deck Expansion (4pk)	99900203-1EA-EU
Opentrons Flex [™] Deck Expansion	99100148-1EA-EU
Opentrons® Tube racks (4-in-1 Set)	99900030-1EA-EU
Opentrons® 1000 µL tip refill, filtered	99100109-20EA-EU
Opentrons® 1000 µL tip refill, filtered	99100112-20EA-EU
Opentrons Flex™ HEPA/UV Module	99900197-1EA-EU
Opentrons Flex™ HEPA Filter Replacement Kit	99900222-1EA-EU
Opentrons Flex™ Thermocycler Gaskets	99100089-1EA-EU
Opentrons Flex™ Waste Chute	99900227-1EA-EU
AAW™ 1-Channel Pipette, 50 μL	99900248-1EA-EU
AAW™ 1-Channel Pipette, 1000 µL	99900246-1EA-EU
AAW™ 8-Channel Pipette, 50 μL	99900247-1EA-EU
AAW™ 8-Channel Pipette, 1000 µL	99900245-1EA-EU
AAW™ Flex Gripper	99900244-1EA-EU
Opentrons® Flex 96-Channel Pipette	99900196-1EA-EU

Description		Catalogue Number
Replacement Power Cord	EU	99100034-1EA-EU
	US	99100028-1EA-EU
	UK	99100038-1EA-EU
	IT	99100036-1EA-EU
	IN	99100031-1EA-EU
	IL	99100035-1EA-EU
	JP	99100029-1EA-EU
	CH	99100037-1EA-EU
	AU	99100032-1EA-EU
	CN	99100033-1EA-EU
	ZA	99100030-1EA-EU

Glossary

This glossary defines terms as they are related to the AAW™ Workstation. It omits industry-standard terms like "labware".

- **96-channel mounting plate** A metal frame that mounts to the z-axis carriage. It holds the 96-channel pipette to the gantry.
- **A1 expansion slot** The area of the deck behind slot A1. When its cover is removed, the A1 expansion slot provides enough space to install the Thermocycler Module. See <u>Deck and Working Area on page 21</u>.
- above deck Space that is on or above the level of the robot's deck area.
- aluminum block See thermal block.
- **ambient lighting** LEDs that illuminate the interior of AAW™, which you can toggle on and off from the touchscreen or the Opentrons® App.
- **auxiliary ports** Ports on the back of the AAW[™] Workstation labeled AUX-1 and AUX-2. The port connection type is an IEC M12 metric screw connector. See <u>Connections on page 32</u>.
- **below deck** The empty area below the robot's deck slots. This space provides clearance for module caddies that sit below the deck and allows for below-deck cable routing.
- caddy See module caddy.
- **calibration pin** A metal pin you attach to the gripper's jaws during gripper calibration. See <u>Calibration</u> on page 30.
- **calibration probe** A metal collar you attach to the nozzle of a pipette during pipette calibration, module calibration, and Labware Position Check. See the Pipette Calibration section in the System Description chapter and the Module Calibration section in the Modules chapter. See also <u>Labware Position Check on page 77</u>.
- **calibration square** The central part of a removable deck slot or module calibration adapter. The square is made of steel to reduce the chance of surface damage during calibration.
- camera A built-in camera that provides an above-deck view inside the AAW™ Workstation enclosure.
- **carrying handle** One of four aluminum handles that screw into the bottom corners of the robot. The handles make the AAW[™] workstation easier to lift. Lifting the robot requires two people. Using the handles is the best way to pick-up/move the AAW[™] Workstation.
- **carrying handle cap** A flat metal cover that goes over the attachment point for a carrying handle. The caps close the handle openings in the frame and give the robot a clean appearance. See Physical Components on page 20.
- **dashboard** The main screen for the robot, accessible by tapping the robot's name in the top left corner of the touchscreen. The dashboard gives you quick access to recently run protocols. See Touchscreen Operation on page 72.
- **deck** The machined aluminum surface on which automated science protocols are executed. It includes the working area, staging area, and A1 expansion slot. See <u>Deck and Working Area on page 21</u>.
- **deck border** The fixed portion of the deck around the four edges of the robot (outside of the area where deck slot panels fit). It contains the removable accessory covers.

- **deck fixture** Hardware items that replace standard deck slots. They let you customize the deck layout and add functionality to your AAW[™] Workstation. Deck fixtures include the staging area slots, trash bin, and waste chute.
- **deck slot** A detachable panel on the deck area. Remove deck slots to install modules and for access to the space below the deck.
- **ejector** The mechanism that automatically pushes tips off the nozzle of a pipette. See <u>Pipettes on page 26</u>.
- **Emergency Stop Pendant** An external accessory that you press to stop the robot immediately. Also referred to as the E-stop. See <u>Emergency Stop Pendant on page 31</u>.
- expansion slot See A1 expansion slot.
- **extension mount** The attachment point on the gantry for the AAW™ Flex Gripper. See <u>Gantry on page 20</u>.
- **finishing cap** See carrying handle cap.
- **firmware** The low-level software that controls the AAW[™] robot and all of its peripheral systems. The firmware is automatically updated on connected instruments and modules to stay in sync with the robot software version.
- fixture See deck fixture.
- frame The outer metal structure of the robot.
- front door The hinged clear panel on the front of the robot.
- **gantry** The robot's positioning system that moves attached instruments horizontally (on the x- and y-axis). See <u>Gantry on page 20</u>.
- gripper An instrument that picks up labware and moves it around the deck automatically.
- home gantry The act of moving the gantry to a defined position at the back right of the working area.
- **instrument** Any component that attaches to the gantry and manipulates liquids or labware on the deck. Examples include the 1-, 8-, and 96-channel pipettes, and the gripper.
- **instrument mount** Attachment point for an instrument. Examples include the pipette mounts and the extension mount for the gripper. See <u>Gantry on page 20</u>.
- **jaws** The moving pincers of the gripper. See <u>Specifications on page 30</u>.
- **JSON protocol** A standardized scientific procedure written as a JavaScript object notation file. The AAW™ Protocol Designer outputs JSON protocols.
- **JSON schema** A set of requirements for the structure and contents of a JavaScript object notation file. For example, all of the labware definitions are formatted according to a particular JSON schema, while JSON protocols follow another schema.
- labware clips The plastic pieces at the corners of deck slots. Labware clips hold labware in place.
- **Labware Creator** The Labware Creator is a no-code, web-based tool that uses a graphical interface to help you create a labware definition file to import into the Opentrons® App. After importing it, custom labware is available to the AAW™ robot and the Python Protocol API.

- **Labware Library** The Labware Library lists the durable and consumable items that can be used with the AAW™ Workstation by default, without customization. It includes things like well plates, reservoirs, tips, tip racks, and tubes.
- **labware offset** Positional data that is created and stored by running Labware Position Check. The AAW™ Workstation takes these offsets into account when moving to a particular type of labware in a particular deck slot.
- **Labware Position Check** A guided process to visually check and adjust pipette movement relative to a piece of labware, with a resolution of 0.1 mm. See <u>Labware Position Check on page 77</u>.
- lift handles See carrying handles.
- lights See ambient lighting or status light.
- **maintenance position** A specific gantry position at the front left side of the working area. The gantry moves to this position to facilitate adding or removing instruments.
- **module** A peripheral that occupies a deck slot. Most modules are controlled by the robot via a USB connection. The Heater-Shaker, Temperature Module, and Thermocycler are all powered modules. The Magnetic Block is an unpowered module. See Modules on page 35
- **module caddy** A container that holds a module. It is used to attach modules to the deck area and help with module removal. Caddies place your labware closer to the deck surface and allow for below-deck cable routing.
- **module calibration adapter** An adapter that sits on top of a module and is used to automatically calibrate module position.
- mounting plate See 96-channel mounting plate.
- **nozzle** The working end of a pipette. AAW[™] pipettes pick up disposable tips by pressing the nozzles down into them. See <u>Pipettes on page 26</u>.
- **Opentrons® App** Software used to control a AAW™ Workstation (or other Opentrons® robots) from a laptop or desktop computer. The Opentrons® App is available for Mac, Windows, and Linux. See Opentrons® App section of the Software and Operation chapter.
- **OT-2** An earlier version of the Opentrons® robot technology, some modules, protocols and programing of the OT-2 can be used with the Opentrons Flex™ and AAW™ Workstations.
- **paddle** Part of the gripper that grasps and holds labware. Paddles are replaceable wear items. See <u>Specifications on page 30</u>.
- **pinned protocol** Protocols you have saved for easy access at the top of the All Protocols tab on the touchscreen. See Protocol management on page 25.
- **pipette** Pipettes are configurable devices used to move liquids throughout the working area during the execution of protocols. There are several AAW[™] pipettes, which can handle volumes from 1 μL to 1000 μL in 1, 8, or 96 channels. See <u>Pipettes on page 26</u>.
- pipette mount The attachment point on the gantry for a pipette. See Gantry on page 20.
- **profile** See Thermocycler profile.
- **protocol** An automated task or procedure to run on the robot. You can also search for, download, and use ready-made protocols from the Opentrons® Protocol Library.

- **Protocol Designer** A web-based, no-code tool for developing JSON protocols that run on robots, including the AAW™ Workstation. See <u>Protocol Designer on page 64</u>.
- **Protocol Library** A public, searchable library that hosts protocols authored Opentrons® or by members of the community. See Protocol Development on page 61 and https://protocols.opentrons.com.
- **protocol run** A particular instance of the AAW[™] Workstation performing the actions specified in a protocol file. Only a single protocol run can be active at any given time. The AAW[™] Workstation stores historical data on the time and outcome of the 20 most recent protocol runs.
- **Python protocol** A protocol script written using the AAW[™] Python Protocol API. See <u>Writing and Running Scripts on page 68</u>.
- **Python Protocol API** A Python package that exposes a wide range of liquid handling features on AAW[™] robots. See <u>Python API on page 56</u> and the online Opentrons[®] Python Protocol API documentation.
- removable deck slot See deck slot.
- run See protocol run.
- **side covers** Detachable panels on the side of the robot, used for module exhaust and external cable routing. See <u>Connections on page 32</u>.
- **side windows** Fixed clear panels on the right and left sides of the robot.
- **staging area** The right-hand side of the deck (column 4), which is only accessible by the gripper. This area requires special staging area slots for use. See <u>Staging Area on page 21</u>.
- **staging area slot** Staging area slots are ANSI/SLAS compatible deck pieces that replace the standard slots in column 3 (A3 to D3) and extend a new slot into the staging area. You can install a single slot or a maximum of four slots to create a new column (A4 to D4) along the right side of the deck. See Staging Area on page 21.
- **status light** A strip of color LEDs along the top front of the robot. This light provides at-a-glance information about the robot. Different colors and patterns of illumination can communicate various success, failure, or idle states. See Touchscreen and LED Displays on page 25.
- **SSH** In programming, SSH stands for Secure Shell, a network protocol that provides a secure channel for accessing and managing remote systems. It's like a secure "remote terminal" that allows you to execute commands on a remote server from your local machine, while encrypting the communication to protect sensitive information.
- **thermal adapter** Aluminum blocks that attach to the Heater-Shaker and hold labware. See <u>Thermal</u>
 Adapters on page 37.
- **thermal block** Aluminum blocks that attach to the Temperature Module and hold labware to facilitate heating, cooling, and maintaining temperature. See <u>Thermal Blocks on page 40</u>.
- **Thermocycler profile** A sequence of temperature changes used by the Thermocycler to perform heat-sensitive reactions. See <u>Thermocycler profiles on page 42</u>.
- **tip rack adapter** An aluminum bracket used by the 96-channel pipette to attach a full rack of pipette tips. See <u>Pipettes on page 26</u>.
- **touchscreen** The interactive LCD screen mounted to the front of the robot. See <u>Touchscreen and LED</u> <u>Displays on page 25.</u>

- trash bin A removable trash container. By default, it occupies slot A3 on the deck.
- tube block See thermal block.
- **USB ports** Connections for accessories, modules, and computers. See <u>USB and Auxiliary Connections</u> on page 32.
- **User Kit** A box that contains tools, fasteners, and spare parts. Every AAW™ Workstation ships with a User Kit.
- waste chute A deck fixture that transfers liquids, tips, tip racks, and well plates from the AAW™
 Workstation enclosure to a trash receptacle placed below its external opening.
- working area The physical space above the deck that is accessible for pipetting. See <u>Deck and Working Area on page 21</u>.
- workstation AAW™ Workstation includes the robot, instruments, modules, accessories, and labware needed to automate a particular application. See <u>Introduction on page 6</u>.
- **z-axis carriage** The gantry component that includes the pipette mounts and the extension mount for the gripper. It moves these instruments along the z-axis (up and down) to locate them precisely during protocol execution. See <u>Gantry on page 20</u>.



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