

# SMC® Human CTLA-4 High Sensitivity Immunoassay Kit

## Microparticle Assay

Human CTLA-4 Immunoassay Kit for the Quantitative Determination of CTLA-4 in Human Serum and Plasma

**03-0220-00**

Introduction .....	2	Assay Reading .....	14
Supplies .....	3	To read on the SMCxPRO® or FemtoQuest™ System.....	14
Included with the Kit.....	3	SMCxPRO® Assay Characteristics ...	15
Kit Storage.....	4	Graph of Typical Reference Curve	15
Additional Supplies Required .....	4	Sensitivity .....	15
Assay Best Practices .....	6	Precision .....	16
SMC® Assay Overview.....	7	Cross-Reactivity .....	16
Assay Preparation .....	8	Spike Recovery .....	16
Sample Preparation .....	8	Troubleshooting .....	17
Sample Dilution.....	8	Precautions .....	19
Reagent Preparation .....	9	Hazard Labels .....	19
QC Preparation .....	9	Terms of Sale.....	22
Initial Standard Stock Preparation	9	Notice .....	23
Standard Curve.....	10	Technical Assistance .....	23
Assay Procedure .....	11	Terms and Conditions of Sale .....	23
Target Capture.....	11	Safety Data Sheets (SDS) .....	23
Post-Capture Wash .....	11	Contact Information.....	23
Detection Antibody Incubation.....	12		
Post-Detection Wash .....	12		
Post-Detection Shake.....	12		
Final Aspiration .....	12		
Elution.....	13		

---

## Introduction

Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4) is a protein receptor found in T cells that plays a role in immune checkpoints and the immune response. It exists in both a membrane-bound form and a soluble form with different functions. The membrane-bound form acts by competing with CD28 for binding with CD80 and CD86 to transmit an inhibitory signal to T cells. By doing so, CTLA-4 can downregulate T cell activity. It has been indicated as playing a role in cancers, autoimmune diseases, and other disease states. High levels of CTLA-4 have been associated with cancer progression and autoimmune disease activity while low levels are associated with overactive immune responses and transplant rejection. It is thought that blocking CTLA-4 activity can enhance the body's response to tumor cells. Multiple CTLA-4 inhibiting antibodies have been approved as cancer therapeutics.

The SMC<sup>®</sup> Human CTLA-4 High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure CTLA-4 in human serum and plasma samples. A capture antibody specific for Human CTLA-4 has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the CTLA-4 present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to CTLA-4 that has been captured onto the beads, thus completing the sandwich. Elution buffer is added to dissociate the protein sandwich, releasing the fluor-labeled antibodies. The eluted antibodies are transferred to a SMCxPRO<sup>®</sup> 384-well plate. The plate is loaded into the SMCxPRO<sup>®</sup> or FemtoQuest<sup>™</sup> System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of CTLA-4 present in the sample. The amount of CTLA-4 in unknown samples is interpolated from a standard curve.

# Supplies

## Included with the Kit

The SMC<sup>®</sup> Human CTLA-4 Immunoassay Kit includes all reagents listed below; these components are lot matched and not intended to be used separately. Additional reagents and supplies are required to run this immunoassay, as listed in the next section; Additional Supplies Required (Not provided). This kit and all reagents supplied are for research use only.

All items are shipped with a cold pack unless otherwise stated.

<b>Description</b>	<b>Storage Conditions</b>	<b>Packaging Details</b>	<b>Component Number</b>
Assay Buffer	2-8 °C	2 x 20 mL	02-9951-00
CTLA-4 Coated Beads	2-8 °C	1 x 550 µL	02-2220-00
Standard Diluent	2-8 °C	2 x 20 mL	02-0225-02
CTLA-4 Detection Antibody	2-8 °C	1 x 270 µL	02-1220-00
CTLA-4 Standard	2-8 °C	1 lyophilized vial	02-8220-00
CTLA-4 Quality Control	2-8 °C	1 lyophilized vial	02-6220-00
10X Wash Buffer	2-8 °C	3 x 50 mL	02-0001-03
Buffer D	2-8 °C	1 x 6 mL	02-0446-00
Elution Buffer B	2-8 °C	1 x 5 mL	02-0211-02
SMC <sup>®</sup> 1 Plex Commercial Plate	RT	1 Plate	02-1PCP-00

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

---

## Kit Storage

The SMC<sup>®</sup> Human CTLA-4 High Sensitivity Immunoassay Kit should be stored at 2-8 °C. Discard standards after one use.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup<sup>®</sup> Filter, for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X Wash Buffer should be discarded upon experiment completion.

Proper kit performance can only be guaranteed if the materials are stored properly.

## Additional Supplies Required (Not provided)

Catalogue numbers are provided to purchase products at [SigmaAldrich.com](http://SigmaAldrich.com) or through sales quote, unless otherwise noted.

### Equipment

- SMCxPRO<sup>®</sup> Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- FemtoQuest<sup>™</sup> System for sample acquisition (95-0200-00)
- Orbital microplate shaker for assay plate incubation (e.g., Boekel Scientific<sup>®</sup> Jitterbug<sup>™</sup>)
- BioTek<sup>®</sup> 405<sup>™</sup> TSUV Plate Washer for SMC<sup>®</sup>/MILLIPLEX<sup>®</sup> Technology (95-0004-06)
- Sphere Mag Plate for performing microparticle capture (90-0003-02)
- Rotisserie tube rotator for microparticle suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x g for sample/plate centrifugation
- Microcentrifuge capable of reaching 13,000 x g for reagent/sample centrifugation
- Single channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- 12-channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

---

## Supplies

- Micro-centrifuge tubes for sample preparation and storage
- 1 L Container with cap for wash buffer dilution
- Stericup® Filter, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- MultiScreen®<sub>HTS</sub> BV 96-Well Filter Plate for sample filtration (MSBVN1210)
- 15 mL conical tube with cap for capture bead and Detection Antibody dilution
- 96-well V-bottom plate for assay setup (Fisher Scientific, 14-222-241)
- Axygen™ Microplate Sealing Film and Tapes (Fisher Scientific, 14-222-344)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- 12-Channel Reagent Reservoir (sterile) for standard serial dilution (Argos/Cole Parmer 04395-33)
- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Millex® Syringe Filter, 0.2 µm for Detection Antibody filtration (SLGPR33RS)
- Luer-Lok® Syringe, 5 mL; for Detection Antibody filtration (Fisher Scientific, 14-829-45)
- SMCxPRO® 384-Well Plate with adhesive seal (02-1008-00)
- SMCxPRO® 384-Well Plate, bulk case of 32 (ABB2-00160A)
- Nunc™ aluminum adhesive plate seals (Fisher Scientific, 276014)

## Reagents

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-00)
- De-ionized or distilled water for dilution of 10X Wash Buffer

## Assay Best Practices

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. In addition, proper training as well as instrument maintenance is critical for obtaining optimal results in performing SMC<sup>®</sup> assays. The following notes should be reviewed and understood before the assay is set up.

- Wipe down bench and pipettes with 70% isopropanol before use.
- It is important to allow all reagents to warm to room temperature (RT), 20-25 °C.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation.

**Note:** It is recommended that the standards are prepared as the last step prior to plate setup.

- All washing must be performed with the Wash Buffer provided.
- An orbital microplate shaker for assay plate incubation (example, Boekel Scientific Jitterbug™ Shaker settings #3-5) provide maximal orbital mixing without splashing liquid or causing cross-contamination.
  - Jitterbug™ Shaker setting #3 ~ 750 rpm
  - Jitterbug™ Shaker setting #4 ~ 875 rpm
  - Jitterbug™ Shaker setting #5 ~ 1000 rpm

**Note:** If using different orbital shaker, refer to recommended rpm ranges provided for each incubation step, and adjust speeds as necessary to ensure maximal orbital mixing without splashing liquid or causing cross-contamination.

- As the SMC<sup>®</sup> assay is extremely sensitive to dust particles, do not perform the assay or plate washing under direct airflow.
- Plate must also be protected from light after adding detection.
- After the assay is complete, seal the plate before reading immediately or storing temporarily at 2-8 °C. The SMCxPRO<sup>®</sup> and FemtoQuest™ Systems require the use of aluminum adhesive plate seal.
- It is not recommended to store eluted products from SMC<sup>®</sup> assays overnight at 4 °C or frozen at -80 °C for later reading as performance cannot be guaranteed.
- If SMC<sup>®</sup> Read Plate has been stored at 4 °C, plate should be left at RT for 30 minutes to 1 hour on the benchtop before reading to avoid a rapid increase in temperature within SMC<sup>®</sup> Read Plate wells. Bring to RT then centrifuge the plate at 1,100 x g for 1 minute prior to reading.
- For optimal SMCxPRO<sup>®</sup> Immunoassay System performance, perform ASSIST testing daily (ideally at beginning of the day before assay is prepared).
- For optimal FemtoQuest™ System performance, perform Self-Test daily and SMC<sup>®</sup> Fluorescence Verification Kit monthly.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

## SMC<sup>®</sup> Assay Overview

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100  $\mu$ L of Standard/Quality Control/neat samples and 100  $\mu$ L of Coated Beads to assay plate.
3. Seal and incubate for 2 hours at 25 °C on appropriate microplate incubator/shaker.



2 hours 25 °C

4. After capture incubation, centrifuge assay plate at 1,100 x *g* for 1 minute.
5. Perform Post-Capture Wash.
6. Remove from washer magnet and add 20  $\mu$ L of Detection Antibody per well.
7. Seal assay plate and incubate for 1 hour at 25 °C on microplate incubator/shaker.



1 hour at 25 °C

8. Perform Post-Detection Wash.
9. Seal the assay plate and perform the post-detection shake for 2 minutes on microplate incubator/shaker.
10. Perform the Final Aspiration.
11. Remove from washer magnet, place on sphere magnet and add 10  $\mu$ L of Elution Buffer B to each well.
12. Seal assay plate and incubate for 10 minutes at 25 °C on microplate incubator/shaker.



10 minutes at 25 °C

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

13. Add 10  $\mu\text{L}$  of Buffer D to neutralize the eluted antibody.
14. Transfer 18  $\mu\text{L}$  of neutralized eluate to the SMC<sup>®</sup> Read Plate.
15. Seal SMC<sup>®</sup> Read Plate with aluminum adhesive plate seal for SMCxPRO<sup>®</sup> or FemtoQuest<sup>™</sup> System.
16. Load on SMCxPRO<sup>®</sup> or FemtoQuest<sup>™</sup> System.

## Assay Preparation

### Sample Preparation

Prepare Serum or Plasma samples by one of the following methods:

- **Preferred Method:** If using a filter plate with prefilter: Stack the filter plate on top of a 96-well receptacle plate. Place 250  $\mu\text{L}$  of sample into a filter plate well and spin for  $\geq 10$  minutes at  $1,100 \times g$ .
- If using a microcentrifuge: Centrifuge samples at  $> 13,000 \times g$  for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

### Sample Dilution

No sample dilution is required, it is recommended to run neat serum or plasma. Samples with higher values can be diluted in the provided standard diluent.

If further sample dilution is required, samples can be diluted with the provided Standard Diluent.

## Reagent Preparation

1. Warm all reagents to room temperature (RT) prior to use.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
  - Pour all 3 bottles of 10X Wash Buffer (containing 50 mL each for 150 mL total) into a container capable of holding at least 2 L. Add 1.35 L of deionized water.
  - Mix thoroughly by gentle inversion or with a clean, sterile stir bar.**Note:** 1X Wash Buffer may be filter sterilized (refer to Storage Instructions).
4. Mix CTLA-4 Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for  $\geq 20$  minutes until all beads are resuspended.

## QC Preparation

1. Reconstitute lyophilized QC in 250  $\mu$ L of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes.
  2. Refer to the Certificate of Analysis for the Final QC expected concentration.
  3. To prepare the High QC, dilute 10  $\mu$ L of the CTLA-4 QC in 990  $\mu$ L of Standard Diluent.
  4. To prepare the Low QC, dilute 20  $\mu$ L of the High QC with 480  $\mu$ L of Standard Diluent.
- Note:**
- If a Mid QC is desired, dilute 100
- $\mu$
- L of the High QC with 400
- $\mu$
- L of Standard Diluent.

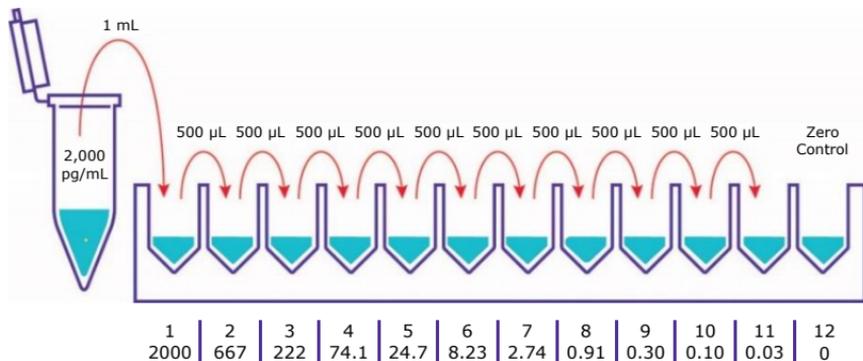
## Initial Standard Stock Preparation

1. Reconstitute lyophilized standard in 250  $\mu$ L of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes.
2. Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the CTLA-4 Standard in the vial.
3. Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 2,000 pg/mL in a 1.0 mL final volume.

## Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the 2,000 pg/mL Standard 1 for Standards 2 through 11 to achieve a curve from 2,000 pg/mL to 0.03 pg/mL. Standard 12 is the Blank (Standard Diluent only).

Run the standards in triplicate.



**Note:** Pipette gently into reservoir wells to avoid creating bubbles.

1. Add 1,000 µL Standard Diluent to wells 2 through 12 of a 12-channel reservoir dilution plate.
2. Transfer 500 µL of 2,000 pg/mL working stock (Standard 1) into well 1.
3. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

---

# Assay Procedure

## Target Capture

1. Pipette 100  $\mu\text{L}$  per well of Standards, Quality Controls, or neat Samples to assay plate.
2. Retrieve the CTLA-4 Coated Bead vial from the rotator and transfer its full contents to 11.0 mL of supplied Assay Buffer. Rinse the bead vial with 0.55 mL of fresh Assay Buffer and ensure that all beads have been transferred from the original vial. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted CTLA-4 Coated Beads.
3. Using a multichannel pipette, add 100  $\mu\text{L}$  per well of diluted CTLA-4 Coated Beads into the assay plate.
4. Seal the assay plate with clear adhesive plate seal, applying pressure to the seal to prevent leaking and cross-contamination.
5. Incubate for 2 hours at 25 °C on microplate incubator/shaker set at approximately 650-875 rpm or Jitterbug™ setting #3.
6. A minimum of 10 minutes prior to the end of target capture incubation, prepare the CTLA-4 Detection Antibody working stock:  
  
Prepare 1X Detection Antibody by adding 250  $\mu\text{L}$  of 20X Detection Antibody into 4,750  $\mu\text{L}$  of Assay Buffer and filter the diluted Detection Antibody using the syringe with a 0.2  $\mu\text{m}$  filter into a clean tube.
7. When incubation is complete, centrifuge the assay plate at 1,100  $\times g$  for 1 minute, place the plate on the washer magnet, and carefully remove clear adhesive plate seal to avoid splashing.

## Post-Capture Wash

Wash plate once with a plate washer using the Post Capture Wash (POSTCAP) program on the Bio-Tek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

## Detection Antibody Incubation

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Using a multichannel pipette, dispense 20  $\mu$ L per well of CTLA-4 Detection Antibody using reverse pipetting without disturbing the bead pellets.
3. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate for 1 hour at 25 °C on microplate incubator/shaker set at approximately 1000 rpm or Jitterbug™ setting #5. Ensure plate is protected from light during this incubation.
5. When incubation is complete, centrifuge at 1,100  $\times$  g for 1 minute then carefully remove the clear adhesive plate seal to avoid splashing.

## Post-Detection Wash

Wash the assay plate with wash buffer using the 4 cycle Pre-Transfer (4CYCPRE) program on the Bio-Tek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

## Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200  $\mu$ L of wash buffer.
2. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
3. Place the plate on the microplate/incubator shaker set at approximately 500-750 rpm or Jitterbug™ setting #3 for 2 minutes. Ensure plate is protected from light during this incubation.
4. Remove the plate from the shaker, and centrifuge at 1,100  $\times$  g for 1 minute. Carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

## Final Aspiration

Perform the Final Aspirate program (FINASP) program on the Bio-Tek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

---

## Elution

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Dispense 10  $\mu$ L Elution Buffer B per well using reverse pipetting without disturbing the bead pellet.
3. Seal assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate the plate for 10 minutes at 25 °C on microplate incubator/shaker set at 1000-1500 rpm or Jitterbug™ setting #5. **Ensure plate is protected from light during this incubation.**
5. When incubation is complete, centrifuge at 1,100  $\times g$  for 1 minute.

## Assay Reading

### To read on the SMCxPRO® or FemtoQuest™ System

1. Place the assay plate with Elution Buffer B onto the sphere mag plate and allow beads to form a tight pellet for 2 minutes.
2. Keeping the assay plate on the magnet, carefully remove the adhesive plate seal. Using a multichannel pipette, add 10  $\mu$ L of Buffer D to center of wells containing Elution Buffer B. Use a fresh tip with each dispense.
3. Set a manual 12-channel pipette to 18  $\mu$ L and put 12 tips onto the pipettor. Transfer 18  $\mu$ L of neutralized eluate solution per well to corresponding wells of the SMC® Read Plate, placed over the included plate holder by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal the 384-well SMC® Read Plate with new clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 x *g*. Remove the seal, inspect SMC® Read Plate wells and remove bubbles if they are present.
5. Firmly seal the SMC® Read Plate with aluminum plate seal using the recommend plate roller.
6. Remove the plate holder from the sealed reading plate and load it onto the SMCxPRO® or FemtoQuest™ System. Start read.

#### **Note:**

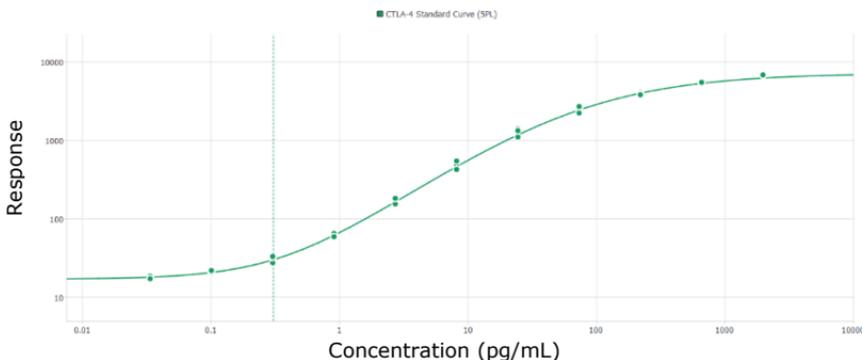
For SMCxPRO® System: There is a warmup period of up to 30 minutes to wait for the SMC® Read Plate to be close to the internal instrument temperature. Once achieved the read will start automatically.

For FemtoQuest™ System: The Immunoassay System will wait (up to 30 minutes) to allow SMC® Read Plate to equilibrate to internal instrument temperature. The 'Status' message, 'Waiting' will be displayed. Once the instrument is ready to read plate, status will change from 'Waiting' to 'Moving to Well' to 'Well Scanning'.

# SMCxPRO<sup>®</sup> Assay Characteristics

## Graph of Typical Reference Curve

Typical SMCxPRO<sup>®</sup> Human CTLA-4 Immunoassay Standard Curve, not to be used to calculate data.



## Sensitivity

Assay sensitivity measures the true limit of quantitation of an analyte and is often defined by the Lower Limit of Quantification (LLOQ). LLOQ is calculated as the lowest concentration that can achieve CVs of < 20% and the percent recovery of the standard point is still between 80%-120%. The LLOQ of CTLA-4 is 0.30 pg/mL.

Please note that the published LLOQ is data generated during kit verification and can have minor variation between kit lots. For lot specific LLOQ, please see the certificate of analysis.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

## Precision

The assay variations of SMC<sup>®</sup> Human CTLA-4 Immunoassay kits were studied using five serum and plasma samples run in triplicate by 3 different operators on 3 different days.

- Mean intra-assay variation was < 10%.
- Mean inter-assay variation was < 20%.

## Cross-Reactivity

Cross-reactivity to the following analytes were tested with the following results:

- Human PD-1: < 1%
- Human PD-L1: < 1%

Specificity to the following species samples were tested with the follow results:

- Mouse: 4 out of 4 individuals were quantifiable.
- Rat: 2 out of 4 individuals were quantifiable.
- Cynomolgus Monkey: 2 out of 2 individuals were quantifiable.
- Rhesus Monkey: 2 out of 2 individuals were quantifiable.
- Canine: 2 out of 4 individuals were quantifiable.
- Feline: 2 out of 4 individuals were quantifiable.

**Note:** Assay has not been verified for the mentioned species.

## Spike Recovery

The data represent mean percent recovery of three different concentrations of standard spiked into samples (n = 5 serum samples, 5 plasma samples).

If you have both separate them out, data should match with verification report.

<b>Sample ID</b>	<b>Serum Recovery</b>	<b>Plasma Recovery</b>
Sample 1	128%	105%
Sample 2	128%	107%
Sample 3	132%	130%
Sample 4	121%	113%
Sample 5	123%	122%
Average	127%	116%

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

## Troubleshooting

<b>Problem</b>	<b>Probable Cause</b>	<b>Solution</b>
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including wash buffer) are not contaminated.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Plate was over-incubated	Confirm plate incubation times are as recommended, particularly for the Detection incubation.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 $\mu$ L or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing (~650-1500 rpm).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x <i>g</i> for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.  Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

<b>Problem</b>	<b>Probable Cause</b>	<b>Solution</b>
Beads are lost during the wash	Plate washer needs optimization/cleaning	Contact Tech Support or local Specialist to schedule washer programming. Refer to user guide for cleaning procedure.
	Insufficiently primed washer	Washer should be primed with wash buffer prior to running the post capture wash protocol.
	Beads came in contact with water	Washer should be primed with wash buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles.
	Proper magnet was not used	Ensure that the mag plate (90-0003-02) was present on plate wash stage prior to running wash protocol.
Published LLoQ was not achieved	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Check plate washer to confirm no beads were lost during washes and that plate contains < 2 µL following the post-capture and final aspiration protocols.  Ensure standards are prepared before starting capture incubation.
Microparticles do not resuspend into homogenous solution	Beads were not properly stored and may have been frozen	Labelled microparticles should be stored at 4 °C. If microparticles are frozen, they will not resuspend properly.
	Samples may be causing interference due to excess particulate matter	Samples should be properly processed prior to testing to remove particulate matter or lipids.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

## Precautions

Use caution when handling biological samples. Wear protective clothing and gloves.

Components of this reagent kit contain sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

## Hazard Labels

Ingredient	Catalogue No.	Label
CTLA-4 Standard CTLA-4 Quality Control	02-8220-00 02-6220-00	 <p><b>Warning.</b> Harmful if swallowed, in contact with skin or if inhaled. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/protective clothing. <b>IF SWALLOWED:</b> Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. <b>IF ON SKIN:</b> Wash with plenty of water. Call a POISON CENTER/doctor if you feel unwell. <b>IF INHALED:</b> Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. Get medical advice/attention if you feel unwell. Take off contaminated clothing and wash it before reuse. Dispose of contents/container to an approved waste disposal plant.</p>
CTLA-4 Coated Beads	02-2220-00	No Label Required
		Harmful to aquatic life. Avoid release to the environment. Dispose of contents/container to an approved waste disposal plant.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

Ingredient	Catalogue No.	Label	
Assay Buffer	02-9951-00	No Label Required	Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.
Standard Diluent	02-0225-02		<b>Warning.</b> May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Do not breathe dust/fume/ gas/mist/vapours/spray. Get medical advice/attention if you feel unwell. Dispose of contents/container to an approved waste disposal plant.
10X Wash Buffer	02-0001-03		<b>Warning.</b> Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Elution Buffer B	02-0211-02	No Label Required	Harmful to aquatic life. Avoid release to the environment. Dispose of contents/container to an approved waste disposal plant.

For research use only. Not for use in diagnostic procedures.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
B	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
C	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
D	QC1	QC1	QC1	QC2	QC2	QC2	QC3	QC3	QC3	Sample 1	Sample 1	Sample 1
E	Sample 2	Sample 2	Sample 2	Sample 3	Sample 3	Sample 3	Etc.	Etc.	Etc.			
F												
G												
H												

For research use only. Not for use in diagnostic procedures.

---

## Terms of Sale

THIS PRODUCT IS INTENDED FOR RESEARCH USE ONLY. YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR THE ENTITY PURCHASING THE PRODUCT, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THESE TERMS OF SALE.

"Product" means FemtoQuest™ Instrument, 95-0200-00, 70-0200-00.

"Commercial Product" means (i) any product intended for sale; (ii) any product intended for use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

"Research Use" means any internal in vitro research use. Such research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the manufacture or sale of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product to provide a service to any third party

PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access. If Purchaser wishes to transfer the Product to a site other than its original site of installation, Purchaser must contact us at the email address below.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

ANYONE INTERESTED IN PURCHASING OR USING THE PRODUCT FOR PURPOSES OTHER THAN RESEARCH USE MUST CONTACT [limitedcommercial@milliporesigma.com](mailto:limitedcommercial@milliporesigma.com) AND AGREE TO SEPARATE TERMS OF LIMITED COMMERCIAL USE PRIOR TO USE OR PURCHASE.

---

## 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 Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice).

### Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at [SigmaAldrich.com/terms](https://SigmaAldrich.com/terms).

### Safety Data Sheets (SDS)

Safety Data Sheets are available on the product page at [SigmaAldrich.com](https://SigmaAldrich.com).

### Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://SigmaAldrich.com/offices).

---

The life science business of Merck operates  
as MilliporeSigma in the U.S. and Canada.

Merck, SMCxPRO, Stericup, Multiscreen, FemtoQuest, Millipore and Sigma-Aldrich are trademarks of Merck or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2025 Merck and/or its affiliates.  
All Rights Reserved.

For research use only. Not for use in diagnostic procedures.

23205554 Rev 10/25

The Merck logo is displayed in a bold, blue, sans-serif font. The letters are closely spaced and have a slightly irregular, hand-drawn appearance.