User Guide

DirectLoad™ Horizontal Electrophoresis System

DMINI
DMIDI

Introduction

The DirectLoad™ Horizontal Electrophoresis System offers a wide degree of versatility for DNA and RNA agarose gel electrophoresis. Multiple tray options are available allowing the choice of gel length options at the time of purchase. Maximizing comb and tray options allow for high versatility of sample loading to be resolved per gel. The total run length allows restriction fragments or other close MW sample bands to be easily separated and identified. Speed loading is accomplished using multi-channel pipette-compatible combs.

For research use only.

Safety Sheet

Before using the DirectLoad™ Horizontal Electrophoresis System, read the printed Safety Sheet included in the product packaging. The Safety Sheet is also available for download, from the product page at SigmaAldrich.com.

Kit Contents

Component	DirectLoad™ Horizontal Electrophoresis System Mini - (DMINI) Size / Detail	Qty	DirectLoad™ Horizontal Electrophoresis System Midi - (DMIDI) Size / Detail	Qty
Tank, including wired electrodes		1		1
Lid		1		1
Trays	(1) 7 x 7 cm, and (1) 7 x 10 cm	2 total	(1) 15 x 7 cm, (1) 15 x 10 cm, and (1) 15 x 15 cm	3 total
Gel tray dams	Mini	2	Midi	2
Combs	1 mm, 8 sample	2	1 mm, 20 sample	2
Loading guides, strips, platform		1		1
DirectLoad™ Electrophoresis cables	(1) Black and (1) Red	1 set	(1) Black and (1) Red	1 set
Safety Sheet, includes operating temperature range and additional product specificiations		1		1

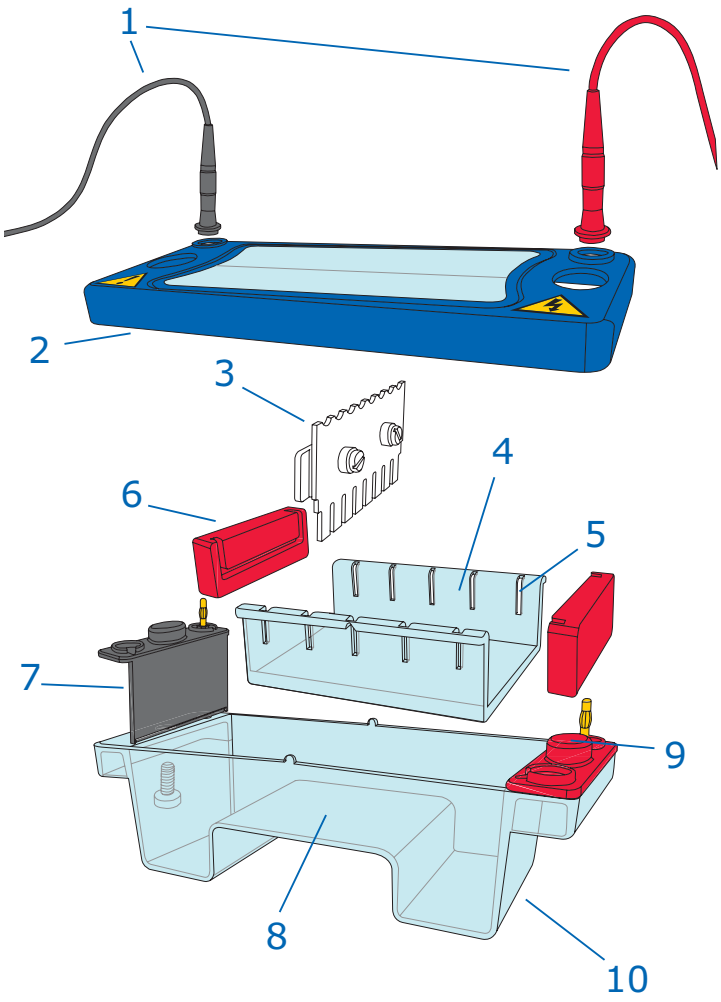
Supplies Needed

(not provided)

- Pipettes
- Tips
- Agarose
- Dyes
- Buffers
- Samples

Components

	Description	Detail or Material
1	Power supply cables	1000V rated with retractable 4 mm connectors
2	Safety lid and viewing pane	Molded Acrylic
3	Height adjustable combs	High Impact Molded Acrylic
4	UV transparent gel tray	Molded Acrylic, UV transparent above 300 nm
5	Comb Slots	Allow multiple combs per tray
6	Casting dams	Red rubber
7	Color-coded electrodes	Molded Acrylic, 99.99% platinum wire, brass and beryllium copper plated 4 mm plugs
8	Gel platform	Notched to fix gel position
9	Safety lid thumb locators	For safe removal of lid
10	Molded buffer tank	Molded Acrylic
	Not Shown	
	DirectLoad™ Mini Loading Guides	optional viewing guides
	DirectLoad™ Mini Viewing Platform	optional viewing guide



Operating Instructions

Setting up the Horizontal Gel Tanks

Fitting Electrodes

1. Note the position of the lid on the base. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the base.

   Note: if the lid is not removed, fitting the cables may result in loosening of the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

Fitting Loading Guides (optional)
These can be fitted to enhance visibility of the wells if desired. They can be fitted to the white vinyl platform sheet or to the device itself.

1. Seat the tray in the device and note the position of the comb grooves. The samples run black to red but the trays can be used frontward or backwards so ensure that the comb grooves closest to the black electrode are marked.
2. Remove the tray.
3. Peel the back off the loading guide and carefully apply the loading guide directly to the gel platform.

Gel Preparation

The table below shows the volume of agarose solution required to make the desired agarose gel for each unit tray size. For a standard 0.7% agarose gel, add 0.7 grams of agarose to 100 mL of 1x TAE or TBE solution. The same 1X solution should be used in the tank buffer solution.

Mini	Tray size	7 x 7 cm	7 x 10 cm	--
	Gel volume for 5mm thick gel	25 mL	35 mL	--
Midi	Tray size	15 x 7 cm	15 x 10 cm	15 x 15 cm
	Gel volume for 5mm thick gel	52.5 mL	75 mL	112.5 mL

1. Add the agarose powder to a conical flask.
2. Add the appropriate amount of 1x TAE or TBE solution from the table above. To prevent evaporation during the dissolving steps below, the conical flask should be covered with parafilm.
3. Dissolve the agarose powder by heating the agarose either on a magnetic hot plate with stirring bar or in a microwave oven. If using the microwave method, the microwave should be set at around a 400 watt or medium setting and the flask swirled every minute. The solution should be heated until all crystals are dissolved. This is best viewed against a light background. Crystals appear as translucent crystals. These will interfere with sample migration if not completely dissolved.

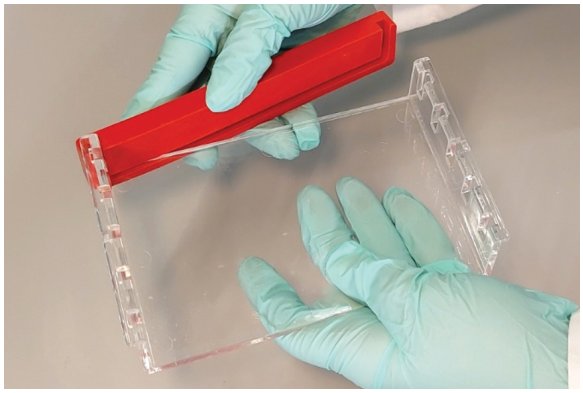
WARNING: The gel must be cooled to 50-60 °C before pouring.

Gel Pouring

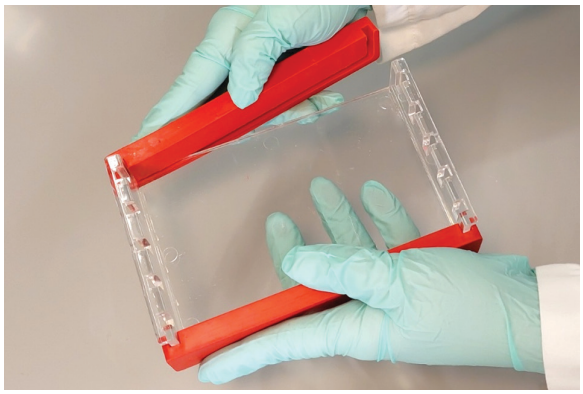
The DirectLoad™ Horizontal Electrophoresis System allows two methods of gel casting:

Casting Dams

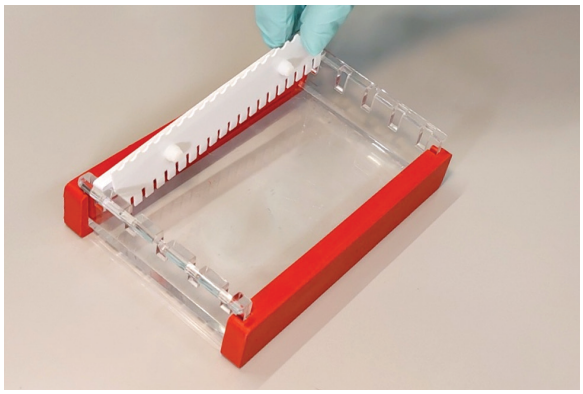
1. To fit the casting dams, place one casting dam with the groove facing gel tray edge
   a. Push the edge of the tray down firmly into the groove.



   b. Repeat this for the other side.



   c. The dams should be fitted so that there is no gap between the sides of the tray and the groove in the dams. This will ensure that there is no possibility of gel leakage.



2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting dams and comb and transfer the gel including tray to the main tank.

Tape

1. Autoclave or plastic backed general tape should be used. A length 5cm longer than the width of each end of the tray should be cut. One length should be placed over one end of the tray and stuck 1 cm in from the tray edge. This should then be folded, and the edges sealed securely. Repeat for the other end and place onto a level surface for gel pouring.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the casting tape and comb, and transfer the gel including tray to the main tank.

Running the Gel

1. Mix the sample to be loaded with loading dye, see Suggested Solutions (right) for common sample buffers. Follow the loading dye manufacturer's recommendation of use.
2. Fill the unit with buffer until the gel is just flooded with buffer. This will give the fastest resolution times. For enhanced quality of resolution of sample, fill the unit to 5 mm above the gel.
3. Load the samples into the wells using pipettes. Multi-channel (MC) pipettes can be used for loading samples with multi-channel pipette compatible combs, see listing in Product Ordering on page 8 for identification of these.
4. Carefully place the lid on the tank and connect to a power supply.
5. Typically, gels are run at between 90 and 150 volts. Maximum voltages are indicated on the serial badge of each unit. It should be noted that higher voltages generally give faster, but poorer quality, sample resolution.

Gel Staining and Viewing

The DirectLoad™ trays allow staining to be performed without removing the gel from the tray if this is preferred.

1. Transfer the gel to a vessel containing the appropriate volume of 0.5 µg/mL ethidium bromide stain for 15–30 minutes, see solutions for stock stain concentration and adjust to the volume used accordingly. The entire gel should be covered.

   NOTE: Ethidium bromide is a suspected carcinogen and the necessary safety precautions should be taken.
2. De-stain the gel for 10–30 minutes in distilled water again ensuring the gel is completely immersed.
3. Rinse the gel twice for a couple of seconds with distilled water.
4. Transfer the gel to a UV Transilluminator.

The samples will often appear as brighter, clearer bands when photographed or viewed using a gel documentation system. However, if the gel bands are too faint then the staining procedure should be adjusted so that there is less de-staining. If there is too much background, then the staining procedure should be adjusted so that there is more de-staining.

Suggested Solutions (sold separately)

For ordering information, see Product Ordering on page 8.

0.5M EDTA Stock (500 mL)
Dissolve in 400 mL distilled water:

93.05g EDTA disodium salt

Fill to 500 mL litre final volume with distilled water

50X TAE Stock (1 L)
Dissolve in 750 mL distilled water:

- 242 g tris base (FW = 121)
- 57.1 mL glacial acetic acid
- 100 mL 0.5 M EDTA (pH 8.0)

Fill to 1 litre final volume with distilled water

10X TBE Stock (1 L)
Dissolve in 750 mL distilled water:

- 108 g tris base (FW = 121)
- 55 g boric acid (FW = 61.8)
- 40 mL 0.5 M EDTA (pH 8.0)

Fill to 1 litre final volume with distilled water

Loading Dye
10x sample buffer stock consists of 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanole FF in 1x TAE buffer. Only 1–10 mL of the 10x loading dye should be prepared.

Ethidium Bromide Solution
Add 10 mg of Ethidium Bromide to 1 mL distilled water.

Care and Maintenance

Cleaning

- Only clean with warm water with a mild concentration of soap or other mild detergent. Water at temperatures above 60 °C can cause damage to the device and components. Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons.
- The tank should be thoroughly rinsed with warm water or distilled water to prevent build-up of salts, but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised.
- Air drying is preferred.
- Do not soak in detergents for more than 30 minutes.
- Do not allow DirectLoad™ Horizontal Electrophoresis Systems to come in contact with the following cleaning agents. They will cause irreversible and accumulative damage
  - Acetone
  - Phenol
  - Chloroform
  - Carbon tetrachloride
  - Methanol
  - Ethanol
  - Isopropyl alcohol
  - Alkalis

RNAse Decontamination

1. Clean the device with a mild detergent as described above.
2. Wash with 3% hydrogen peroxide (H_2O_2) for 10 minutes.
3. Rinsed with 0.1% DEPC-(diethyl pyro carbonate) treated distilled water.

   Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using.

   RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Storage

See Environmental Conditions in the Safety Sheet provided in the product packaging. It can also be downloaded from the product page at SigmaAldrich.com.

End Of Life Instructions WEEE Directive

In accordance with European Union directive on the management of waste electrical and electronic equipment (WEEE), this product must not be disposed of in unsorted municipal waste at the end of its life. It must be taken to a collection and recycling center. For further information, go to SigmaAldrich.com/WEEE.

Troubleshooting

Problem	Cause	Solution
Bands sharp but not enough bands seen	Gel agarose percentage too high	Decrease agarose percentage.
	Incomplete digestion	Review enzyme activity, digest further.
Band smearing and streaking	Agarose has improper endosmosis	Reduce salt concentration to ≤0.1 M.
	Salt concentration in sample too high	
	Excessive power and heating	Reduce voltage. See Running the Gel on page 5.
	Sample spilled out of well	Apply sample carefully. Increase gel thickness for large sample volumes. Adjust comb height.
	Incomplete digestion, nuclease contamination, bad enzyme	Heat sample. Review enzyme activity. Digest sample further.
	Sample wells cast through the gel. Sample leaks along bottom of running surface	Comb should be placed to 1-2 mm above the base of the running surface.
Curved line or distortion of bands	Bubbles in sample wells	Remove bubbles prior to electrophoresis.
Curved bands, smiles	Sample overload	Reduce load.
Differential relative mobilities	Sample spilled out of wells	Samples should have proper density. Sample may contain ethanol or phenol, insure the sample is free of contaminants before loading it onto a gel.
	Unit not levelled	Level unit. Use a steady work bench.
Gels crack	Too high voltage gradient, especially with low melting temperature agarose or low gel strength gels	Reduce voltage. Run gel at lower temperature.
High MW bands sharp; low MW bands smeared	Gel agarose percentage too low	Increase agarose percentage. Switch to polyacrylamide.
Ragged bands	Sample density incorrect	See Running the Gel on page 5 for sample preparation prior to loading onto a gel.
	Sample well deformed	Carefully remove comb, especially from soft gels. Make sure gel has solidified.
	Excessive power or heating	Cooling soft gels aids in comb removal.
		Reduce voltage. See Running the Gel on page 5 for electrophoresis instructions.
Slanted lanes (bands)	Gel not fully solidified	Gel to solidify for at least 30-45 minutes.
	Comb warped or at an angle	Check alignment of comb.

Product Ordering

Order products online at SigmaAldrich.com.

DirectLoad™ Mini Horizontal Electrophoresis System — DMINI

with (1) 7 x 7 cm
and (1) 7 x 10 cm gel trays

Description	Size	Catalogue Number
DirectLoad™ Mini Loading Guides		
Loading Guides	mini	DMS7-LG
DirectLoad™ Mini Tank Accessories		
Lid	mini	DMS7-LID
Negative Electrode	mini	DMS7-NE
Positive Electrode	mini	DMS7-PE
Tank (Including Electrodes)	mini	DMS7-TANK
DirectLoad™ Electrophoresis cables (Black & Red)	both	DMS-CABLES
DirectLoad™ Mini Combs		
8 sample	1 mm	DMS7-8-1
10 sample	1 mm	DMS7-10-1
12 sample MC	1 mm	DMS7-12-1
16 sample	1 mm	DMS7-16-1
8 sample	1.5 mm	DMS7-8-15
12 sample MC	1.5 mm	DMS7-12-15
16 sample	1.5 mm	DMS7-16-15
8 sample	2 mm	DMS7-8-2
DirectLoad™ Mini Gel Trays		
Mini Gel Tray Dams	mini	DMS7-DAMS
7 x 10 cm	mini	DMS7-TR10
7 x 7 cm	mini	DMS7-TR7

Note: "MC" identifies multi-channel, pipette-compatible combs.

DirectLoad™ Midi Horizontal Electrophoresis System — DMIDI

with (1) 15 x 7 cm, (1) 15 x 10 cm
and (1) 15 x 15 cm gel trays

Description	Size	Catalogue Number
DirectLoad™ Midi Loading Guides		
Loading Guides	midi	DMS15-LG
DirectLoad™ Midi Tank Accessories		
Lid	midi	DMS15-LID
Negative Electrode	midi	DMS15-NE
Positive Electrode	midi	DMS15-PE
Tank (Including Electrodes)	midi	DMS15-TANK
DirectLoad™ Electrophoresis cables (Black & Red)	both	DMS-CABLES
DirectLoad™ Midi Combs		
16 sample MC	1 mm	DMS15-16-1
20 sample	1 mm	DMS15-20-1
30 sample MC	1 mm	DMS15-30-1
28 sample MC	1.5 mm	DMS15-28-15
28 sample MC	1 mm	DMS15-28-1
20 sample	1.5 mm	DMS15-20-15
35 sample	1 mm	DMS15-35-1
16 sample MC	1.5 mm	DMS15-16-15
10 sample	1 mm	DMS15-10-1
DirectLoad™ Midi Gel Trays		
Midi Gel Tray Dams	midi	DMS15-DAMS
15 x 10 cm	midi	DMS15-TR10
15 x 15 cm	midi	DMS15-TR15
15 x 7 cm	midi	DMS15-TR7

Additional Supplies

RNaseZAP™ cleaning agent for removing RNase	R2020

References

1. Sambrook, Fritsch, and Maniatis, Molecular Cloning A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989.
2. Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, 1989.

Notice

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