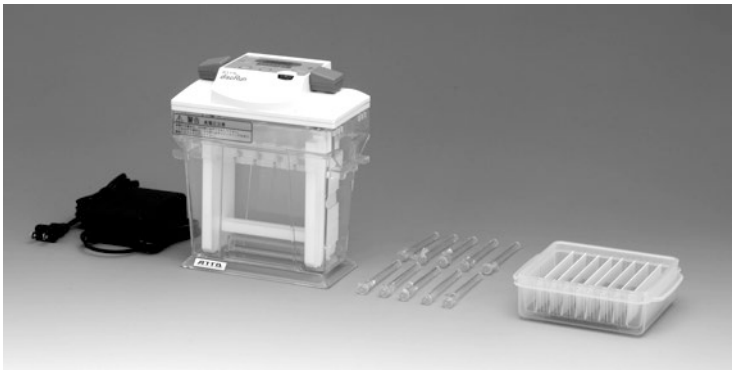
AE-6541 Disc Gel EP Kit (discRun)
AE-6540 Disc Gel EP Kit



Operation Manual

Feb. 2005
Version 1.2
ATTO Corporation

Index

1. General Information

1.1. Introduction

This Manual describes how to use the ATTO AE-6541 discRun rod gel electrophoresis system. The explanation omits the basic experimental procedures of molecular biology and biochemistry, so please refer to the references and bibliography entries before conducting experiments.

1.2. Purpose of use

AE-6541 discRun is IEF electrophoresis system for the 1st dimension of 2-dimensional electrophoresis.

1.3. Warnings and Cautions

To ensure safe use:

Precautions are provided to prevent incorrect usage, and thereby ensure that there are no accidents (however unlikely) when using this product. Be sure to carefully read precautions and operate so there are no accidents.

⚠Warning: indicates a potentially hazardous situation, which, if not avoided, could result in death or serious injury.

⚠Caution: indicates a potentially hazardous situation, which, if not avoided, may result minor or major injury or damage the apparatus.

- Do not touch the (electrophoresis) apparatus except for control panel during the power supply is on.
- Do not operate the instrument with wet hands.
- Do not operate the instrument in extreme humidity (above 95%). If wetted, unplug the power supply until the instrument is dry. Let the electrical equipments such as plug and connector dry completely before use.

2. Specifications

2.1. Components (AE-6541MM/MC/B, AE-6540)

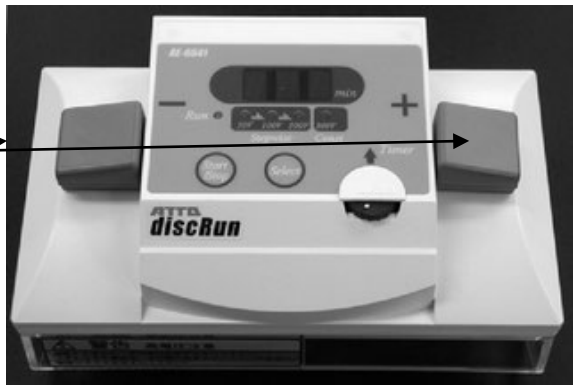
	AE-6451MM/MC (For hand-cast gel)	AE-6541B (For agarGEL precast gel)	AE-6540
Power supply module	1	1	--
AC adapter	1	1	--
Safety lid	--	--	1
Lead wire	--	--	1 pair
Upper chamber	1	1	1
Lower chamber	1	1	1
Seal gasket	8	8	8
Silicone cap No.2	7	7	7
Column rods	8	--	--
Membrane fixing rings	8	--	--
Membrane	0.3 m	--	--
Rod gel casting syringe	1	--	--
Mini-Compact gel tray	1	1	1
Consisting of:			
Outer tray	1	1	1
Inner tray	1	1	1
Cover lid	1	1	1
Base plate	1	1	1
Separating plate	8	8	8
Gel carrier	1	1	1
Column cleaning blush	1	--	--
Operation Manual	1	1	1

2.1.1 Power supply module

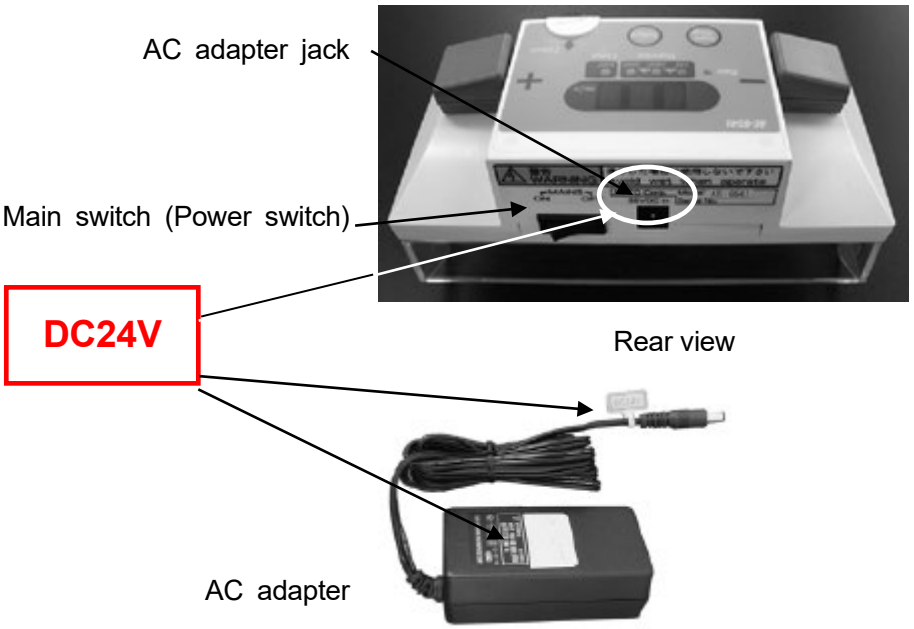
Operation panel

Electrode connectors

Push to connect to the electrodes on the upper chamber. Pull to disconnect them. Always the right knob is anode (+) and the left knob is cathode (-).



Front view



Caution: The input voltage for the power module is changed from 36VDC to 24VDC on February 2005. Old (S/N 421144 or earlier) and new AC adapters are not compatible. Please check the AC adapter for voltage and make sure to use collect one.

2.1.2. Operation panel

(1) Timer
Set value and running time is displayed in minutes as well as the end of electrification and error messages.

(2) Run indicator
This indicator lights when running electrophoresis.

(3) Mode indicator
This indicator shows the selected mode using the “Select” key.

(4) Start/Stop
Press this key to start or stop electrophoresis. The Start/Stop key is effective when the selected mode is “Stepwise, 50V” or “Const., 300V.”

Operation panel

Note: While running electrophoresis and the key is pressed to stop, the elapsed time shown on the timer is reset. Set the time once again to resume.

(5) Mode Select key

Press this key to select mode and then set the timer.

Stepwise, 50V → Stepwise, 100V → Stepwise, 300V → Const, 300V → Stepwise, 50V → …

For agarose IEF

	Time to set (minutes)	
Mode	For compact size gel (50 mm)	For mini size gel (75 mm)
Const, 300V	150	210

For O'Farrell system

	Time to set (minutes)	
Mode	For compact size gel (50 mm)	For mini size gel (75 mm)
Stepwise, 50V	10	10
Stepwise, 100V	10	10
Stepwise, 300V	145	205

(6) Timer dial

Push the dial toward the power supply module to enter time. A period (.) will be indicated at the right bottom of the numbers.

Display

Turn the dial clockwise or counter-clockwise to choose appropriate value to set. To set continuous output, select "-.-.-."

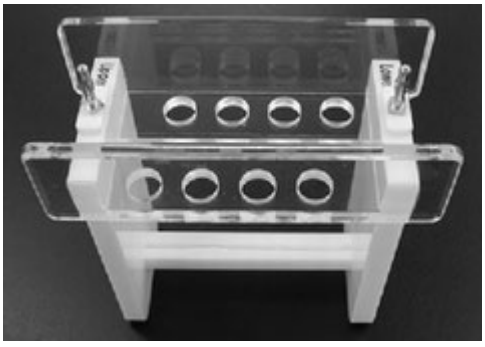
When the dial is pushed while running, currently set time is displayed during it is pushed.

(7) Memory function

The module memorize the selected mode and set time in the previous operation for each mode, and the memory is saved even after power is off.

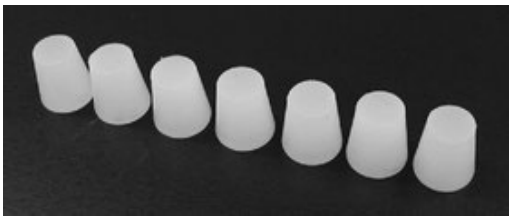
2.1.3 Other parts

Lower chamber

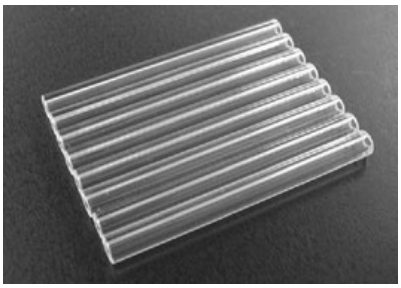


Upper chamber

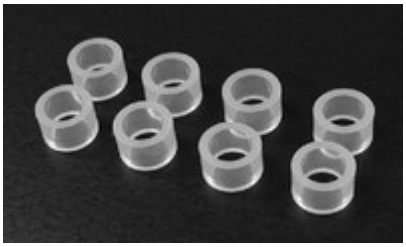
Seal gasket



No. 02 Silicone cap

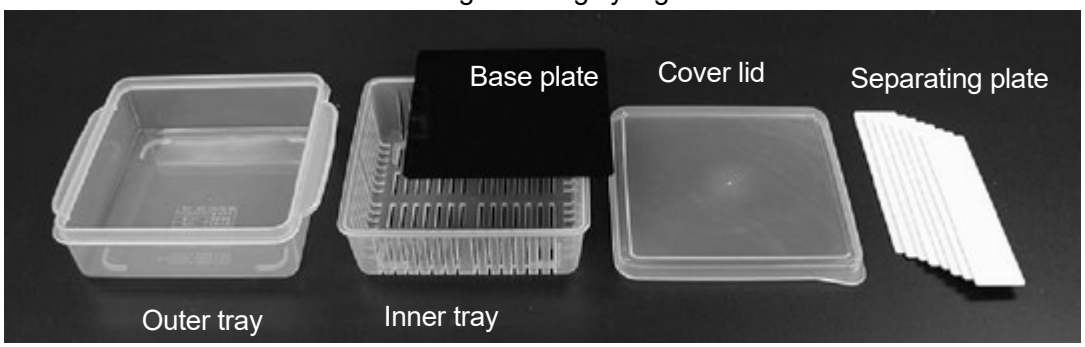


Gel column

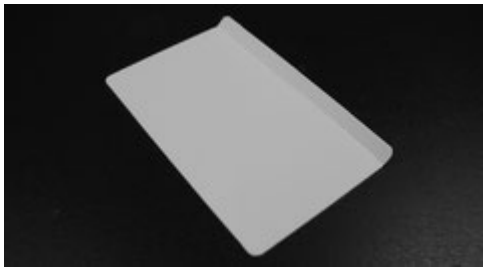


Membrane fixing ring

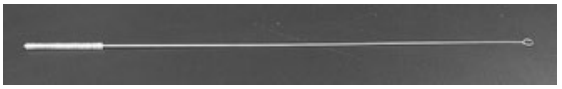
Rod gel casting syringe



Mini Compact gel tray



Gel carrier



Column cleaning brush

Option: agarGEL precast gel (required for AE-6540B)

2.2. Specifications

Number of simultaneous electrophoresis gels: Max. 8
Quantity of buffer: Upper electrode buffer: 40 mL
Lower electrode buffer: 550 mL

Power supply module

Output mode:	Constant voltage
Output voltage:	50, 100, and 300V
Output current:	40 mA max.
Safety functions:	Short/open-circuit detection
Timer:	Continuous/ 1 – 250 min; lapse time display
Alarm:	At time-up and detecting an error

Materials
Upper chamber: Acrylic resins
Lower chamber: Polycarbonates
Power supply case: ABS
Safety cover: Polycarbonates

AC adapter:

Input:	90 – 240 VAC, 50/60 Hz
Output:	24 VDC, 0.7A*

*Note: Products manufactured before Jan. 2005: 36 VDC, 0.6A

Dimensions: 164 mm (W) x 94 mm (D) 193 mm (H), 0.81 kg

Caution: The input voltage for the power module is changed from 36VDC to 24VDC on February 2005. Old (S/N 421144 or earlier) and new AC adapters are not compatible. Please check the AC adapter for voltage and make sure to use collect one.

3. Preparation

3.1 Overview

Agarose 2-dimensional electrophoresis

A standard flow of an experiment of agarose two-dimensional electrophoresis using compact/mini slab gels in the second dimension is shown below.

3.2 Time for preparation

- Preparation of gels for the first dimension (not required when using agarGEL precast gel)
  Approx. 2 hours are required for gel preparation, and 4 hours to overnight for gel solidification.
- Sample preparation (to be stored at -80 degC; never repeat freeze and melt.)
  Approx. 1 hour is required.

3.3 Example of timetable

<u>For gel length of 50 mm (Compact size gel)</u>

Operation	Time	Duration
Preparation	09:00 – 09:15	15 min.
Isoelectric electrophoresis, 1st dimension	09:15 – 11:45	2.5 hours
Gel preparation, 2nd dimension	09:20 –	
Gel solidification, 1st dimension	11:50 – 11:55	3 min.
Gel cleaning (1), 1st dimension	11:55 – 12:00	3 min.
Gel cleaning (2), 1st dimension	12:00 – 14:00	2 hours
Gel equalizing, 1st dimension	14:00 – 14:10	10 min.
Gel migration, 1st dimension	14:10 – 14:15	5 min.
SDS-PAGE, 2nd dimension	14:15 – 14:50	35 min.
Gel solidification (CBB dyeing), 2nd dimension	14:55 – 15:15	10 min.
Gel dyeing (CBB dyeing), 2nd dimension	15:05 – 15:25	20 min.
Gel decoloring (CBB dyeing), 2nd dimension	15:25 – 15:55	30 min.
Gel storing and image photographing	16:00 –	

<u>For gel length of 75 mm (Mini size gel)</u>

Operation	Time	Duration
Preparation	09:00 – 09:30	30 min.
Isoelectric electrophoresis, 1st dimension	09:30 – 13:30	3.5 hours
Gel preparation, 2nd dimension	09:35 –	
Gel solidification, 1st dimension	13:05 – 13:10	3 min.
Gel cleaning (1), 1st dimension	13:10 – 13:15	3 min.
Gel cleaning (2), 1st dimension	13:15 – 15:15	2 hours
Gel equalizing, 1st dimension	15:15 – 15:25	10 min.

Gel migration, 1st dimension	15:25 – 15:30	5 min.
SDS-PAGE, 2nd dimension	15:30 – 17:00	90 min.
Gel solidification (CBB dyeing), 2nd dimension	17:05 – 17:25	20 min.
Gel dyeing (CBB dyeing), 2nd dimension	17:25 – 18:05	40 min.
Gel decoloring (CBB dyeing), 2nd dimension	18:05 – 19:05	60 min.
Gel storing and image photographing	19:10 –	

⚠Warning: Toxic, dangerous materials and mutagens will be used in electrophoresis experiments. Always ware gloves and eye-goggles to protect the skin.

Reference: Electrophoresis 2000, 21, 1653-1669; “Preparative two-dimensional gel electrophoresis with agarose gels in the first dimension for high molecular proteins.”

3.4 Reagents

Prepare the reagents stated below.

(1) For 1st dimension IEF

- Agarose IEF (Amersham Biosciences)
  Code No.: 27-0468-01 (not required when using Agargel)
- Sorbitol (class 1 or above) (not required when using Agargel)
- Urea (of high purity)
- Thiourea (special class)
- Pharmalyte™ (Amersham Biosciences)
  Code No.: pH 2.5 – 5, 17-0451-01; pH 4 – 6.5, 17-0452-01;
  pH 5 – 8, 17-0453-01; pH 8 – 10.5, 17-0455-01, etc.
  *Select the type of Pharmalyte to be used according to the purpose.
- Complete™ Mini EDTA-free (protease inhibitor cocktail) (Roshe Diagnostics)
  Product No. 1 836 170
- CHAPS (3-Cholamidopropyl Dimethylammonio Propanesulfonic Acid)
  E.g. Wako Pure Chemical Industries, Ltd., Product No. 347-04723
- Triton™ X-100 (polyethyrene glycol mono-p-iso-octylphenyl ether)
  E.g. Wako Pure Chemical Industries, Ltd., Product No. 591-12191
- DTT (dithiothreitol)
- Hydrochloric acid (HCl) (highest grade)
- Sodium hydroxide (NaOH) (highest grade)
- Phosphoric acid (85% solution) or DL-aspartic acid (highest grade)
- Trichloroacetic acid (powdered, highest grade)

- IEF marker, 2-D marker, if needed.

Choose product that can be used with a denaturing system (includes urea). Because the present specification includes urea in the gel, when using markers for an undenatured (native) system, accurate results may not be obtained.

(2) For 2nd dimension SDS-PAGE

- Acrylamide (for electrophoresis)*
- N,N'-Methylene-bisacrylamide (Bis) (for electrophoresis)
- Ammonium persulfate (for electrophoresis)
- N,N,N',N'-tetramethylethylenediamine (TEMED) (for electrophoresis)
- Tris-(hydroxymethyl) aminomethane (Tris) (bio-science grade)
- Sodium dodecyl sulfate (SDS) (bio-science grade)
- HCl (highest grade)
- Bromophenol blue (BPB) (highest grade)
- Agarose (for electrophoresis)
- Molecular weight marker, if needed.

*Note: Acrylamide is not required when e-Pagel/c-Pagel precast gels are used.

(3) For CBB staining

- Acetic acid (highest grade)
- Methanol (highest grade)
- Coomassie Brilliant Blue (CBB) R-250 or G-250 (for electrophoresis)

4. Preparation for buffers

(1) Sample buffer (Storable at −20°C for 2 months.)

		Final concentration
Tris	72 mg	(60 mM)
Urea	3.60 g	(5 M)
Thiourea	0.76 g	(1M)
Complete™ Mini EDTA-free	1 tablet	
CHAPS	0.10 g	(1%)
Triton™ X-100	100 μL	(1%)

Dissolve all above in distilled water to make a 10 mL solution, and adjust the pH of the resulting solution at 8.8 to 9.0.

Notes:

- Dissolve DTT (10 mg per 1 mL) immediately prior to sample preparation.
- The pH 8.8 – 9.0 is important for the reduction and modification of thiol groups. Please be sure to follow the pH value.
- Mercaptoethanol is not recommended due to its weak reduction power.
- Sample buffers containing DTT must be stored at −20°C and use within a week.
- Do not heat the sample buffer to 30°C or more. The urea is dissolved and isocyanate is produced. The isocyanate carbamylates proteins. This causes to change pI.
- Optimum composition of sample buffer varies according to the samples. Please try this composition as reference.

(2) Acrylamide or iodoacetamide solution (Storable at −20°C for 2 months.)

		Final concentration
Acrylamide	71 mg	(1M)

Dissolve in 1 mL of distilled water.

Or

Iodoacetamide	185 mg	(1M)

Dissolve in 1 mL of distilled water.

(3) Overlay solution (Storable at −20°C for 2 months.)

		Final concentration
Urea	1.2 g	(2M)

Dissolve in distilled water to make a 10 mL solution.

(4) Upper electrode buffer (Storable at room temperature for 2 months.)

		Final concentration
NaOH	4.0 g	(0.2M)

Dissolve in 500 mL of distilled water.

Caution: Do not exceed the concentration above for sodium hydroxide. It may cause damages for equipment.

(5) Lower electrode buffer (Storable at room temperature for 2 months.)

		Final concentration
DL-aspartic acid	5.32 g	(40 mM)

Dissolve in 1,000 mL of distilled water.

*Do not use L-aspartic acid because pH won't be accurately adjusted due to its low solubility.

Or

Phosphoric acid (85% solution)	680 μL	(10 mM)

Add to 1,000 mL of distilled water.

(6) 1D Gel Solidification Solution (Storable at room temperature for 2 months.)

		Final concentration
Trichloroacetic acid (powder)	12.5 g	(2.5%)

Dissolve in distilled water to make a 500 mL solution.

(7) Solution A (Storable at 4°C for 1 month. Not needed when precast gels are used.)

		Final concentration
Acrylamide	146.0 g	(29.2%)
Bis	2.0 g	(0.8%)

Dissolve in distilled water to make a 500 mL solution.

(8) Solution B (Storable at 4°C for 1 month. Not needed when precast gels are used.)

		Final concentration
Tris	91.0 g	(1.5 M)
SDS	2.0 g	(0.4%)

Dissolve in distilled water, adjust with HCl at pH 8.8 and make a 500 mL solution.

(9) Solution C (Storable at 4°C for 1 month. Not needed when precast gels are used.)

		Final concentration
Tris	6.1 g	(0.5 M)
SDS	0.4 g	(0.4%)

Dissolve in distilled water, adjust with HCl at pH 6.8 and make a 100 mL solution.

(10) Solution D (Storable at 4°C for 1 week. Not needed when precast gels are used.)

		Final concentration
Ammonium persulfate	100 mg	(10%)
SDS	2.0 g	(0.4%)

Dissolve in 0.1 mL of distilled water.

(11) SDS-PAGE electrophoresis buffer (Storable at room temperature for 2 months.)

		Final concentration
Tris	6.0 g	(25 mM)
Glycine	28.8 g	(192 mM)
SDS	2.0 g	(0.1%)

Dissolve in distilled water to make 2,000 mL solution.

(12) 4.12. SDS equilibration buffer (Storable at 4°C for 2 months.)

		Final concentration
Solution C	50 mL	(50 mM)
SDS	10 g	(2%)
BPB	5 mg	(0.001%)

Dissolve in distilled water to make 500 mL solution.

(13) Agarose solution to adhere the 1st dimension gel
(Storable at room temperature for 2 months.)

		Final concentration
Agarose	100 mg	(1%)
SDS-PAGE electrophoresis buffer	10 mL	

Heat and dissolve them and divide the resulting solution in 1 mL portions.

(14) Decolorizing solution (Storable at room temperature for 1 month.)

		Final concentration
Methanol	300 mL	(30%)
Acetic acid	100 mL	(10%)

Dissolve in distilled water to make 1,000 mL solution.

(15) CBB staining solution (Storable at room temperature for 1 month.)

		Final concentration
Methanol	300 mL	(30%)
Acetic acid	100 mL	(10%)
CBB R-250 or G-250	1.0 g	(0.1%)

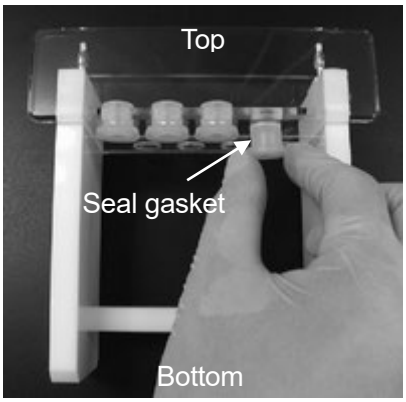
Dissolve in distilled water to make 1,000 mL solution.

5. Gel and Sample Preparation

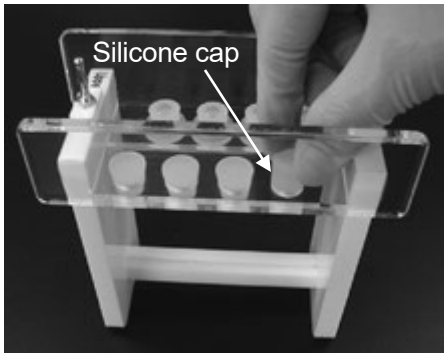
5.1 1st dimension IEF gels

- Ware gloves.
- Sufficiently wash and dry gel columns.

Put seal gaskets in the upper chamber from bottom to top. Push the gasket until it stops.

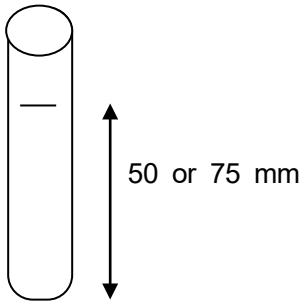


Plug the unused holes of the upper chamber with silicone caps. Push down till it stops.

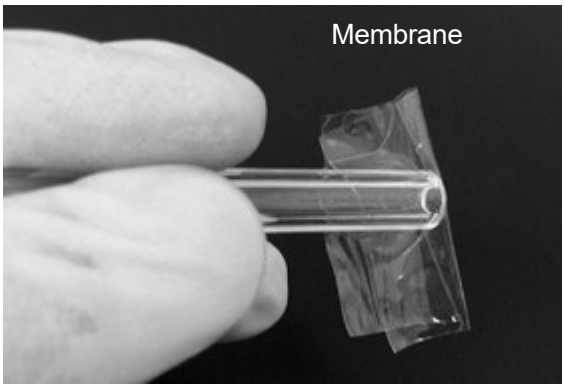


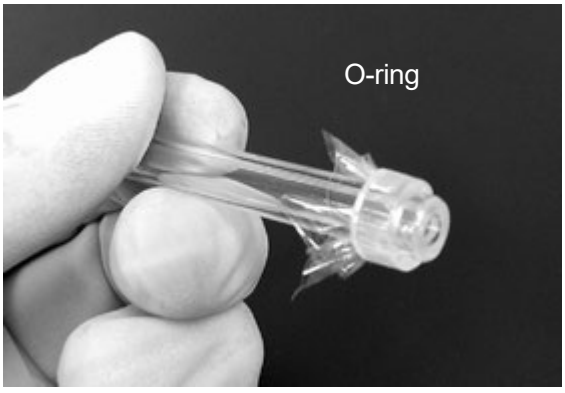
♦ Gel preparation marker

Mark thoroughly washed and dried columns using an oil-based marker 50 mm from one end for the compact size system and 75 mm from one end for the mini-size system.

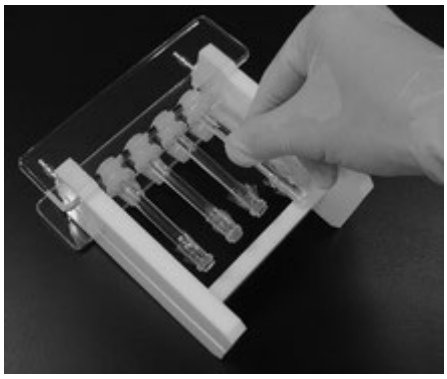


Cut a 3 cm square of membrane. Place it in a distilled water for 1 minutes or more.
Place the membrane on the end of the columns further from the gel preparation marker and fix it with an O-ring.

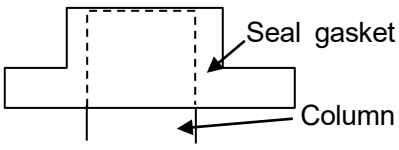




Set the columns on a gel preparation rack (upper chamber).
Insert the column from bottom of the chamber.



Adjust the top of the gel column with the top of the seal gasket. (As shown in the right.)



Set the upper chamber into the lower chamber.

Preparation of Agarose gel solution:

- Weigh and mix the distilled water and reagents for solution A1 below. Gently mix the resulting solution to be uniform.
- Weigh the reagent B1 below.
- Warm the desired volume of Pharmalite™ to room temperature.

<u>For pH 3 – 10, 16 compact size gels</u>

Solution A1	
Distilled water	2.62 mL
Agarose IEF	0.06 g
Sorbitol	0.76 g

Reagent B1	
Urea	1.90 g
Thiourea	0.48 g

Pharmalite™	
pH 2.5 – 5	159 μL
pH 5 – 8	159 μL
pH 8 – 10.5	159 μL

Calculate to adjust the volume according to the number of gels.

Adjust for desired pH range so as total volume of Parmarite being 477 μL.

E.g.) For pH 5 – 10

pH 5 – 8	239 μL	
pH 8 – 10.5	239 μL	Total: 477 μL

E.g.) For pH 5 – 8

pH 5 – 8	477 μL

<u>For pH 3 – 10, 16 minisize gels</u>

Solution A1	
Distilled water	5.24 mL
Agarose IEF	0.13 g
Sorbitol	1.52 g

Reagent B1	
Urea	3.80 g
Thiourea	0.96 g

Pharmalite™	
pH 2.5 – 5	318 μL
pH 5 – 8	318 μL

pH 8 – 10.5 318 μL

Calculate to adjust the volume according to the number of gels.

Adjust for desired pH range so as total volume of Parmarite being 477 μL.

E.g.) For pH 5 – 10

pH 5 – 8	239 μL	
pH 8 – 10.5	239 μL	Total: 477 μL

E.g.) For pH 5 – 8

pH 5 – 8	477 μL

Heat solution A1 in a 100 °C water bath for approximately 20 minutes to completely dissolve the components.

Note: Dissolve until sparkling crystals are not visible. Unsolved Agarose leads to poor electrophoresis patterns.

Once Solution A1 is dissolved, quickly add Reagent B1. Mix the resulting solution, and completely dissolve Reagent B.

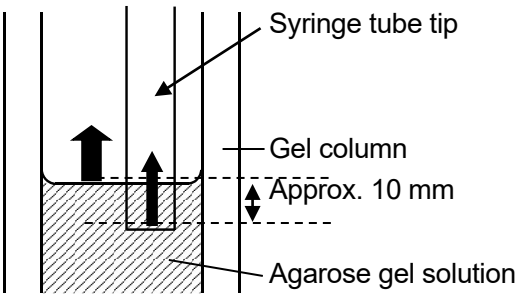
Note: Agarose will be fixed if Reagent B1 is not added quickly.

After complete dissolution, add Pharmalite™ one by one while gently stirring and mix the resulting solution evenly.

If bubbles are seen, leave the resulting solution to stand for several minutes until all bubbles disappear.

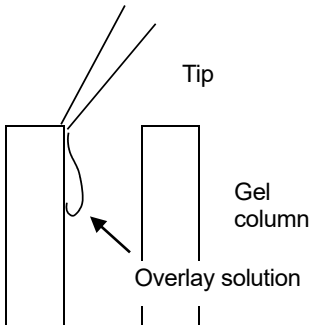
Pour the agarose gel solution into the gel casting syringe, and inject the agarose gel solution into the columns.

Insert the tip of the tube connected to the syringe to the bottom end of each column, and as the liquid surface elevates, bring the tube upward.



After injection, place 50 μL of the overlay solution on top of the gel solution. Using a narrow tip, gradually place the overlay solution along the inner wall of each column.

Note: The overlay solution may clog the gel column if the solution is injected too quickly.



Place plastic wrap over the chamber. Leave the chamber and columns for solidification at 5 – 10 °C for 4 hours to overnignt.

Note: The rod gels can be stored at 5 – 10 °C for up to 5 months. Do not use the gels if they become dry.

5.2 Sample preparation

Dissolve DTT in the sample buffer. (10 mg per 1mL of sample buffer)
Note: Sample buffers containing DTT must be stored at −20 °C and used within 1 week because the reducing power of DTT decreases over time.

Add 5 – 20 volumes of sample buffer to a sample (tissue or cells), and sufficiently dissolve the sample using devices such as a homogenizer at 4 – 10 °C.
If the sample is a solution, add 9 volumes of sample buffer.

Teflon homogenizer

Centrifuge the sample buffer at 50,000 rpm for 30 minutes at 4 °C.
Thiol group is reduced during this 30 minutes.

Recover the supernatant. If there is a lipid layer on the liquid surface, recover the supernatant without aspirating the lipid. Make a hole on the lipid layer by the tip and use a new tip to recover the supernatant.
Note: Lipid contamination can lead to clogging of insoluble components, thus markedly disrupting electrophoresis patterns.
Measure the concentration of proteins as necessary.

Add 1 M acrylamide solution or 1 M iodoacetamide (1/10 the volume of the sample buffer) and mix the resulting solution.
Leave the solution at room temperature for 10 minutes to modify thiol groups.

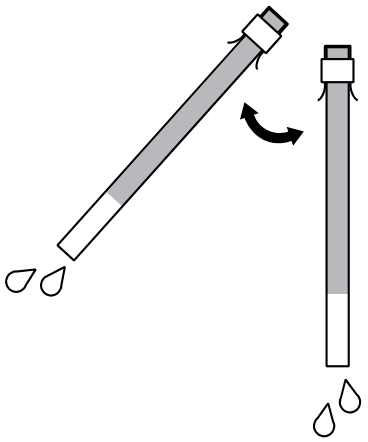
Note: In order to prevent reassembly of disulfide bonds, thiol groups are modified to stabilize. The modification of thiol groups by Acrylamide is referred to as the *Michael* reaction or *Michael* addition.
The reassembly causes erroneous electrophoresis results: homooligomers and heterooligomers can form and marked vertical and horizontal streaking can appear on 2-D patterns.

The sample buffer can be stored at −80 °C for approximately 2 weeks, but using it as soon as possible is recommended for better result.

6. Operation

6.1 1st dimension IEF

Overlay solution is layered on the top of fixed hand-cast rod gel or agarGEL precast gel. Discard the overlay solution with shaking the columns upside down.

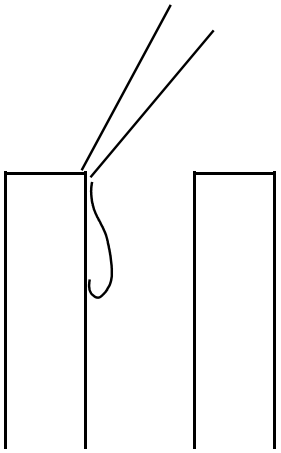


Set the columns on the upper chamber.
Plug the unused holes of the upper chamber with silicone caps.

Pour 500 to 550 mL of the lower electrode solution on the lower chamber.

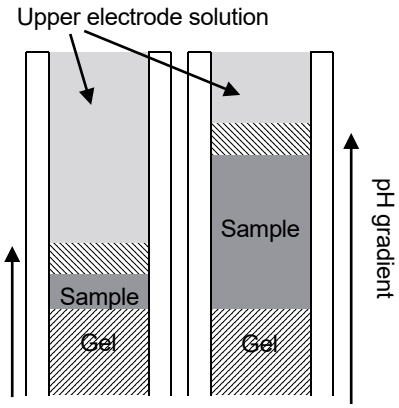
Set the upper chamber into the lower chamber. Apply an appropriate amount of sample solution to the upper end of the first-dimension agarose gel.

If CBB staining is used following second-dimension electrophoresis:
100 μg for the compact-size system (gel length: 50 mm)
200 μg for the mini-size system (gel length: 75 mm)
If silver staining is used following second-dimension electrophoresis:
1 - 2 μg for the compact-size system (gel length: 50 mm)
2 – 4 μg for the mini-size system (gel length: 75 mm)
is a rough standard for the total protein mass.
Use 100 μL or narrower tip to gradually pour the sample along the inner wall of the columns.



The upper electrode solution may clog the gel column if the solution is injected too quickly.

Keep the volume of the sample as little as possible.
50 μL or less is recommended.
The top end of the pH gradation is produced at the boundary surface between the overlay solution and the upper electrode solution. If sample volume is large, pH gradation of the rod gel becomes narrow.

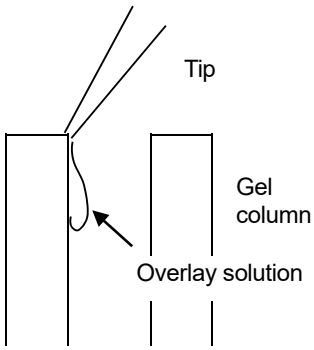


Large sample volume

Place 10μL overlay solution on the sample solution. Use a 100μL or finer scale chip and slowly place the solution by sliding it down the inside wall of the gel column.

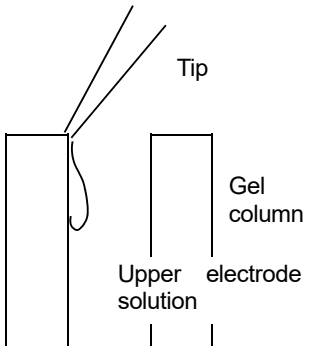
Place the upper electrode solution on the overlay solution up to the top of the gel column. Use a 100μL or finer scale chip and slowly place the solution by sliding it down the inside wall of the gel column.

Look into the gel column directly from above and make sure no air bubbles are present in the column. If present, use the tip of the microsyringe or an injection needle to remove the bubbles.



The overlay solution may clog the gel column or the sample solution surface may be disturbed if the solution is poured too quickly.

Pour 40mL upper electrode solution into the upper vessel. Gently pour so that the electrode solution will not directly hit the top of the gel column.

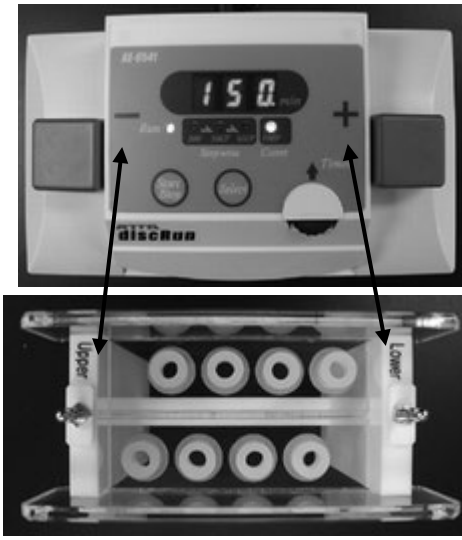


The upper electrode solution may clog the gel column if the solution is poured too quickly.

There are two methods to apply samples: from alkaline side and from acid side. Application from alkaline side is recommended and described as following.

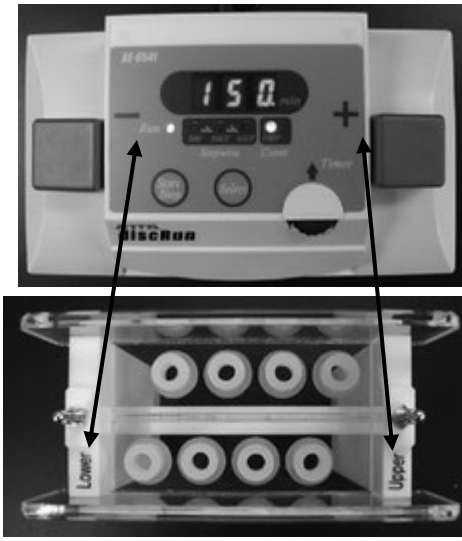
Place the power supply module on the chamber by checking the directionality. The “+” electrode connector of the power supply should match the LOWER electrode terminal on the upper vessel and the “-” connector match the UPPER terminal. Push the “+” and “-” electrode connectors downward.

Note: The upper electrode solution may clog the gel column if the solution is poured too quickly.



“–“ for “Upper” “+” for “Lower”

Application from the acid side is possible by reversing the connection. When using this, replace the upper and lower electrode solutions with each other.



“–“ for “Lower” “+” for “Upper”

Warning: Do not use when the power supply module or electrodes are wet. It may cause an electrical shock or a hardware failure. Stop using this equipment and contact your local distributor or Atto.

Connect the AC adapter to the power supply module.

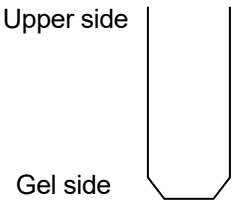
Press Mode Select button to set “Const, 300V.” Push the control dial toward the power supply and turn to set running time. Set 150 minutes for compact size (50 mm) gels or 210 minutes for mini size (75 mm) gels. After setting the running time, press Start/Stop button to start running.

Immediately after the start of electrophoresis, and as the high-molecular-weight proteins start to migrate, the bores on the top of the agarose gel for the first dimension are broken physically, and some constriction is formed. Because of this, polymer proteins are safely separated without being trapped by the gel net.

For the mini and compact disc electrophoresis units without a dedicated power supply, set the safety cover on the electrophoresis chamber, and connect the leads to the customer’s power supply. Electrophoresis lasts 210 minutes for mini-size and 150 minutes for compact size at constant 300V.

(4) Preparation of Gel for the Second Dimension
(This process is not required when using e-Pagel or Pagel Compact.)

Gels for the second dimension are prepared during isoelectric electrophoresis for the first dimension. This process is not required when using e-Pagel or Pagel-Compact. Gels are prepared up to the top end of the cutout using the optional flat comb for mini 2-D and flat comb for compact 2-D.

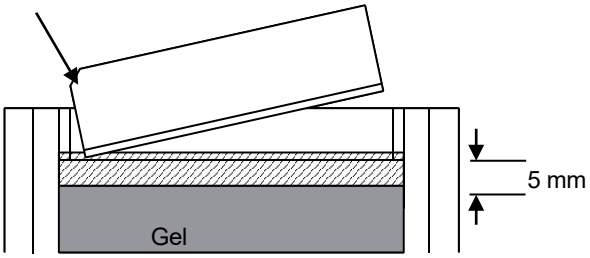


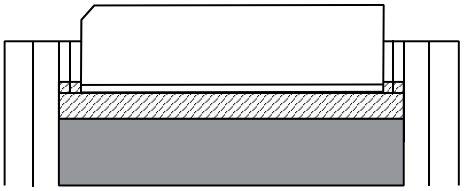
Cross-sectional view of 2-D flat comb

Refer to the instruction manual for your slab type electrophoresis chamber for the detailed description of gel preparation.

Resolving gels are prepared up to approximately 5mm below the cutout on the gel plate. Inject the concentrating gel solution to fill the cutout completely. Insert the 2-D comb obliquely into the cutout on the electrophoretic plate, making sure that the side with the cut comb tip contacts the gel as shown in the figure to the right. Do not forget to remove any air bubbles while inserting the comb.

There are no differences for either left or right this corner will be.



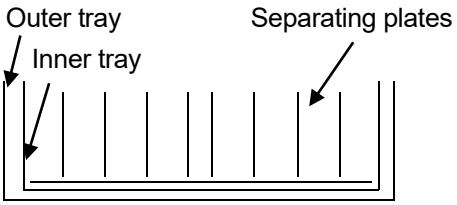


Note: Be sure to remove air bubbles. The electrophoretic pattern may be disturbed severely if air bubbles are not completely removed.
The comb will not turn down as it is supported by surface tension of the gel solutions between comb and flat plate.

(5) Fixing, Cleaning, and Equilibrium of 1-D Gel

Assemble the mini and compact gel tray as shown in the figure to the right and inject 100mL 1-D gel solidification solution.

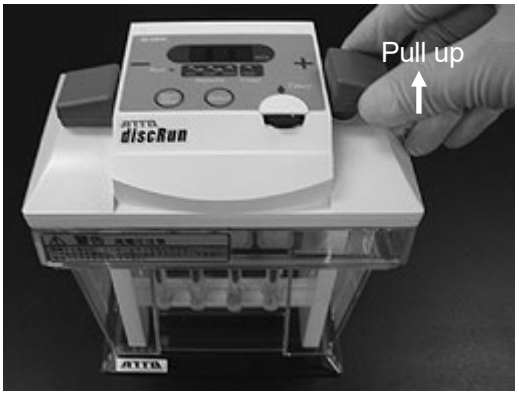
* The 1-D gel solidification solution is strongly acidic. Handle it carefully.



Mini and compact gel tray as assembled

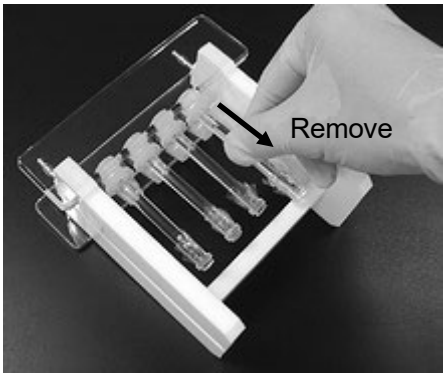
When the 1-D isoelectric electrophoresis has been completed, turn off the main switch on the power supply module and disconnect the AC adapter power plug from the outlet.

Lift off the electrode connectors and remove the power supply module from the lower chamber.

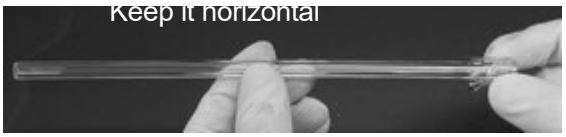


Remove the upper vessel from the lower vessel. Dispose of the upper electrode solution. Wash off residual upper and lower electrode solutions from the upper vessel with distilled water.

Remove the gel column from the packings.



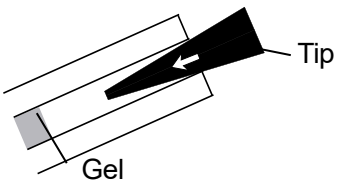
Hold and keep the gel column horizontal and remove the dialysis membrane fixing ring and the dialysis membrane.



On the mini and compact gel tray, tilt the column with the dialysis membrane attached end downward. The 1-D gel slides off from the column. Immerse it slowly in the 1-D gel solidification solution prepared in the tray.

When the gel will not slide off from the column even though you tilt the column, attach a Pasteur pipette rubber cap on the other end of the column and push the gel off from the column.

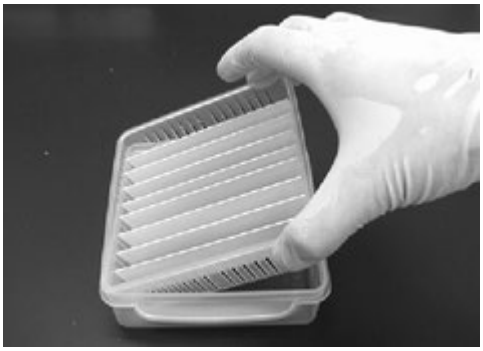
<u>Gels are soft and easily damaged. Do not give strong shocks to them in the steps described below.</u>



Push the gel out by air-pressure.

Shake for three minutes gently.

After 3-minute solidification, remove the inner tray by pulling it gently from the outer tray. Hold the inner tray aslant when pulling it to facilitate drainage of the solution.



Drain the 1-Dgel solidification solution from the outer tray and replace the inner tray in the outer tray. In the steps that follow, remove the inner tray as described above whenever the solution is changed.

Replace the inner tray in the outer tray.
Note: Check the separating plate for not being moved by touching the edge of the outer tray.
Gently pour 100 mL of distilled water into the outer tray and stir for approximately 1 minute.
Then change the water to new one and repeat shaking to wash. Repeat one more time, 3 times in total.

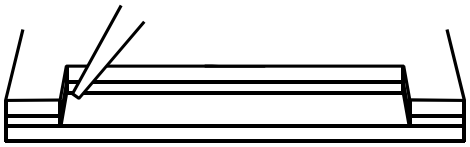
Change the water once again and gently stir for 2 hours.
The gels can be stored 1 to 2 days in the distilled water. If longer storage is necessary, remove the gels from the water, cover them using plastic wrap, and freeze them. The frozen gels can be stored up to 3 month. When using the frozen gels, thaw the gels at room temperature before use.

Discard the distilled water, add 100 mL of SDS equilibration buffer, and gently stir for 10 minutes.

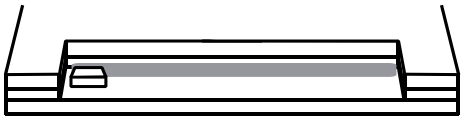
While stirring the gels, soak the molecular weight marker using a square piece of clean filter paper (3 x 3 mm). The guideline of protein mass per band for CBB staining is 200 ng for mini-size system and 100 ng for compact-size system. (1/50 to 1/100 the volume for silver staining.) For 1 mm in thickness and 3 mm square filter paper, the volume of molecular weight marker should be 3 μL or less. Too much marker volume causes poor 2-D pattern. Use 2 pieces of filter paper if the volume is high.

6.2 2nd dimension SDS-PAGE

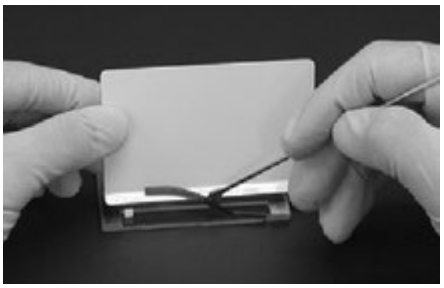
Place the notch of the gel plate upward and forward.



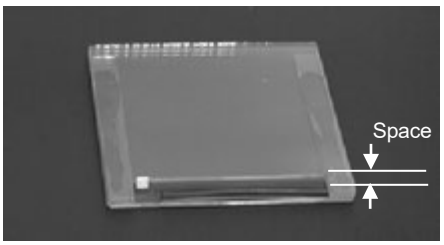
Place a filter paper soaked in the molecular weight marker using forceps.



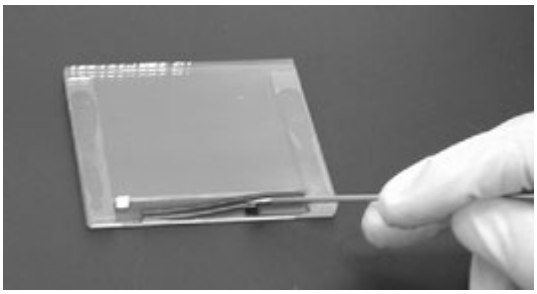
Use gel carrier to place the 1st dimension gel in front of the notch of the plate without damaging the gel.

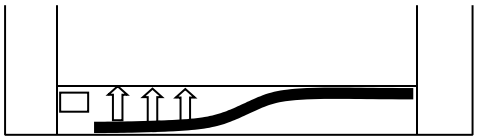


Do not touch the 1st dimension gel to the top of 2nd dimension gel.

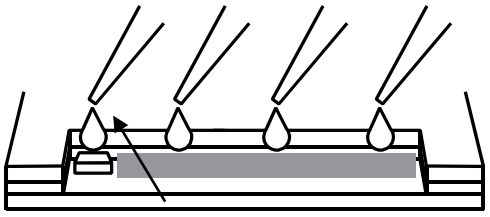


Then carefully adhere the first dimension gel without creating air bubbles.





Heat and dissolve the agarose solution to adhere the first dimension gel, and place several drops at the contact region between the first dimension gel and the upper end of the second dimension gel.
The total volume of the agarose solution is 100 μL for mini-size system and 60 μL for compact-size system.

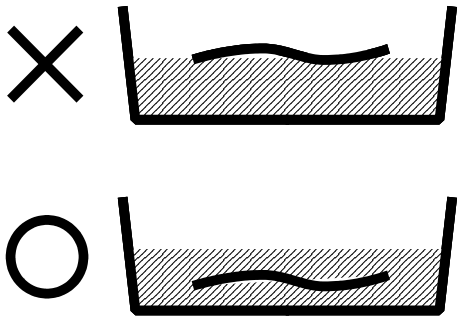


Agarose solution to adhere the 1st dimension gel

Perform 2nd dimensional electrophoresis according to the operation manual of the instrument.

6.3 Staining

6.3.1 CBB stain

Place the gel after 2nd dimensional electrophoresis in decolorizing solution. Stir the gel for 20 minutes for mini-size gel or for 10 minutes for compact-size gel. If this process of fixation is omitted, staining takes long time. Be sure that the gel is in the solution completely. Even staining is not available if the gel floats on the solution surface.	

Discard the decolorizing solution, and stir the gel in CBB staining solution for 40 minutes for mini-size gel or for 20 minutes for compact-size gel.

Discard the staining solution, and stir the gel in fresh decolorizing solution for 60 minutes for mini-size gel or for 30 minutes for compact-size gel. The staining procedure should be completed while a slight amount of background noise remains because longer decolorizing time causes faint spots.

6.3.2 Other staining

For other staining such as silver staining, fluorescent staining and negative staining, follow the protocols described in references and instruction manuals of each stain.

7. Maintenance

Caution: Use of this product may contain use of dangerous carcinogenic reagents. Always wear gloves, and never allow it to directly contact your skin when you clean

these items.
Be sure to disconnect the power code from power outlet before maintenance in order to avoid electric shock.

- Cleaning of equipment
  For materials and equipment used, apply neutral detergent to a soft sponge before drying and wash and dry naturally without leaving fragments of gel or the like. Contact with an acetone-based organic solvent may cause deformation/discoloration.
- Do not rinse or wet the power supply module.

8. List of supplies

2394xxx	Power supply unit for AE-6541, 100-240V	Incl. AC adaptor (100-240V, universal type)
2393720	Safety Cover, MP	Incl. power leads. Use with a separate power supply
2394112	Upper chamber for AE-6541	
2393715	Lower Chamber, MP	Base unit
2392110	Grummets for EP & IEF Tubes 12/pk	Common for electrophoresis & IEF tubes
2392344	Dialysis Membrane	
2393546	Membrane O-Rings 10-7 x 6 mm, 3/pk	
2394122	Mini gel tubes, 7 x 2.5 x 75 mm, 8/pk	
2394132	Mini gel tubes, 7 x 2.5 x 100 mm, 8/pk	
2393365	Silicone caps, 6/pk	
2393423	1st-D Gel Casting Syringe, AE-6310	
2394140	Gel carrier for AE-6541	
2394250	Gel Tray	Incl. plates
2394065	2-D Combs 2/pk, CP	For 2nd dimensional compact gel
2394160	2-D Combs 2/pk, MP	For 2nd dimensional mini gel
Size: 2.5 mm ID x 50 mm, for 2nd dimension EP using compacti PAGE systems		
2332100	A-C310 agarGEL CP, 10/pk	pH 3 - 10
2332110	A-C38 agarGEL CP, 10/pk	pH 3 - 8
2332120	A-C58 agarGEL CP, 10/pk	pH 5 - 8
2332130	A-C510 agarGEL CP, 10/pk	pH 5 - 10
Size: 2.5 mm ID x 75 mm, for 2nd dimension EP using mini PAGE systems		
2332200	A-M310 agarGEL RM, 10/pk	pH 3 - 10
2332210	A-M38 agarGEL RM, 10/pk	pH 3 - 8
2332220	A-M58 agarGEL RM, 10/pk	pH 5 - 8
2332230	A-M510 agarGEL RM, 10/pk	pH 5 - 10

Warranty

Atto Corporation warrants all its products subject to the terms and conditions set forth below.

1. This warranty covers all new products that are sold by Atto Corporation (hereinafter called Atto).

2. Expendable items are not covered by this agreement.

3. Claims under this warranty are limited to defects in material and workmanship of the products.

4. Malfunction and/or damage due to neglect, abuse, operation or repair contrary to specifications and/or instructions presented by Atto are not warranted.

5. Atto shall not be liable to consequential damage, labor, loss or expense directly or indirectly arising from us of the products.

6. Damage due to transit is not covered by this warranty.

7. The warranty period is one (1) calendar year from a date when the products are shipped from Atto to an original purchaser.

8. This warranty is not applied to any defect that is reported to Atto later than one (1) calendar month from a date of warranty termination.

9. Atto Shall supply parts to replace faulty parts of defective products under this warranty, free of charge.

10. Atto shall repair defective products under this warranty which cannot be repaired at field, free of charge.

11. Atto shall replace defective products under this warranty which cannot be repaired, free of charge.

12. Freight charges for return and replacement shipments under this warranty are shared by Atto and a purchaser, that is one way by either party and another way by another party.

13. Warranty period of repaired products and replacement products or parts is three (3) calendar months from a date when the said products or parts are shipped from Atto, or a remaining term of an original warranty period of the defective products, whichever lasts longer.

14. Return of the products for credit or refund is not accepted unless otherwise agreed in writing by Atto.

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