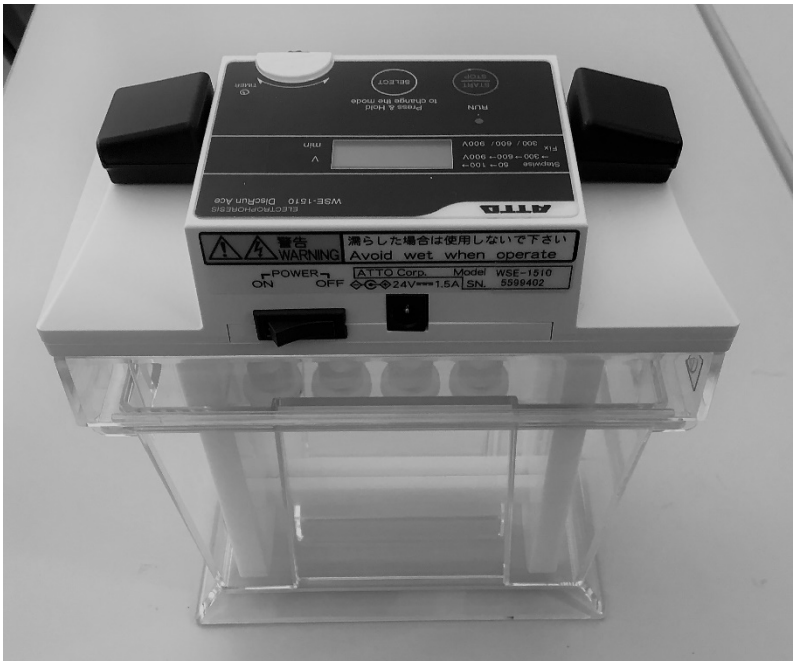
WSE-1510 Disc Run Ace



Operation Manual

4th December 2020
ATTO Corporation

Index

1. General Information

1.1. Introduction

This Manual describes how to use the ATTO WSE-1510 Disc Run Ace 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

WSE-1510 Disc Run Ace is IEF electrophoresis system for the 1st dimension of 2-dimensional electrophoresis.

1.3. Safety Precautions

Correct operation is prerequisite in order to use this instrument safely. Please read through this instruction manual before use, and do not use the instrument until you fully understand the contents. How to use and the safety precautions listed on this manual are specific to usage of this particular instrument only. Never use the instrument for other purposes than those listed on this manual. Any other usages of this instrument than those listed on this manual would be strictly at your own responsibility.

The first-time user is asked to receive guidance from a person with correct knowledge and understand principle and method before use. Both the fresh users and experienced users keep this manual at hand for effective use. The only way to avoid electric shock and malfunction of the unit is to operate it correctly in accordance with this manual.

Feel free to contact us for any questions about principle of electrophoresis, operation, maintenance and inspection.

Safety Notation

The following signs are used on this instruction manual for your safe use and maintenance of the instrument. Please ensure your understanding of the meaning of these signs and follow each item correctly.

Symbol	Description
	This indicates that a high risk of human death or serious injury is likely occurring if handled incorrectly or by neglecting this indication.
	This indicates that human death or injury is possible if handled incorrectly or by neglecting this indication.
	This indicates that the risk of property damage is expected if mishandled or by neglecting this indication.

Symbol	Description
	This indicates information related to the important portion.
	This indicates some hint related to operation.
	This indicates prohibited activities.

Use Precautions

These are precaution items to prevent fire, electrical shock, other type of accident and failure. Please be sure to follow and understand these items.

Danger

Power connection 	Please check visually if any deformation or corrosion exists on an electrode terminal or power plug, any blemish on the power cable, or any peel-off of the insulating sheath before actuating the unit. These might cause fire because of poor contact or electrical shock. In such a case, please contact us after stopping the use of the unit and unplugging the power plug from the outlet. Be sure to turn off the power switch, firmly hold the power plug itself, and unplug it without pulling the cable.
No wet hand 	Never operate the unit with wet hands. In addition, never touch the power plug or connecting terminal with wet hands. These can cause electrical shock or failure. Never use the unit in wet condition. These can cause electrical shock or failure.
Main body 	Never remove the top lid of the main body nor the rear panel. These can cause electrical shock or failure.
Maintenance 	Please quit using the unit immediately if anything abnormal happens during unit usage or if any abnormality/failure is suspected. Also, do not use the unit if you find any failure at inspection. These can cause electrical shocks or damage to the unit. Contact us if you detect any abnormality, trouble or failure.

Warning

Installation location 	Please avoid installation on places such as a wobbling table, tilted location, or heavily vibrating place. Install the unit on horizontal places with safe and hard surfaces, such as a lab bench.
Main body 	This unit is not an explosion-proof structure. Install this unit away from any places with the possibility of exposure to fire or flammable gasses.
Migration 	Never move the unit during operation. Cords may get entangled, which might cause rollover. Or, cords may be damaged, which might cause fire or electrical shock. Please make sure to turn the unit power switch OFF, and pull out the power cable and all wiring cables before migration.
Maintenance 	Please be sure to turn the unit power switch OFF and pull out the power cable before performing maintenance and cleaning. These might cause electrical shock. Please ask us for periodical maintenance, inspection and calibration so as to maintain good performance and safety of the unit.
Disassemble prohibition 	Never disassemble or modify the unit. Adjustment and repair work of the product should be done by our service staff in charge. When adjustment or repair is required, please ask our company. Any accident caused by your disassembling or modification would be outside of our responsibility.
Seal group 	Never peel off the Warning Seal. The seals indicate dangerous areas. If any seal comes off, please contact us.
AC adapter 	Do not use the AC adapter of this device for any purpose other than the operation of this device. It may cause a malfunction or accident. We are not responsible for any accidents or failures caused by using the AC adapter of this device other than this device. Non-standard adapter If you

AC adapter 	use, there is a risk of overheating and ignition. If you have any questions, please contact us or your distributor.
	If you use an AC adapter other than the one provided, the device, power supply, output failure or damage, deterioration or damage of the AC adapter, or ignition may occur. When using this device, be sure to use the AC adapter that came with this device.

Caution

Dew condensation 	When the unit is quickly moved from a low-temperature area (low-temp. room, cold outdoor, etc.,) to a high-temperature area (warm room, etc.,), there is possibility of [Condensation], i.e. moisture in the air becomes water drops. If condensation happens within the unit, some parts may short out. When the unit is moved from a low-temperature area to a high- temperature area, set the unit where there is plenty of ventilation and away from direct sunlight, and turn the unit power on AFTER the unit gets to the same temperature as the room temperature.
Application 	This device is a research physics and chemistry device. Since it is not a medical device, it cannot be used for medical treatment such as medical judgment and confirmation of the effect of treatment.
Export 	Export of specific work and cargo are controlled by Foreign Exchange Laws and Cabinet Order/Ministerial Orders of Foreign Trade Control Laws and those controls are applied to this unit. Even if the unit is not applicable to the Cabinet Order, it is required to submit documents accordingly and if it is applicable, then obtain export license from the Ministry of Economy, Trade and Industry, and then submit the license to the customs office. When you export our product, please confirm with your supplier or our customer service department in advance.
Trademark / Copyright 	You are hereby notified that any distribution, copying or forwarding of this manual is strictly prohibited without permission of ATTO Corporation. Information in this manual or specification of the product is subject to change without notice.

Notice

Seals 	Never peel off nameplate seals. These indicate important information for maintenance and control of the unit.
Note 	Do not touch the (electrophoresis) apparatus except for control panel during the power supply is on.
Note 	Do not operate the instrument with wet hands.
Note 	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 (WSE-1510)

	WSE-1510 Disc Run Ace
Power supply module	1
AC adapter	1
Upper chamber	1
Lower chamber	1
Grummets for EP & IEF Tubes	8
Silicone cap No.2	7
Agar gel tray	1
Consisting of:	
Outer tray	1
Inner tray	1
Cover lid	1
Base plate	1
Separating plate	7
Gel carrier	1
Operation Manual	1
Option:	
Gel column	-
Membrane fixing rings	-
Membrane	-
Rod gel casting syringe	-
Column cleaning blush	-

2.1.1 Power supply module

Operation panel

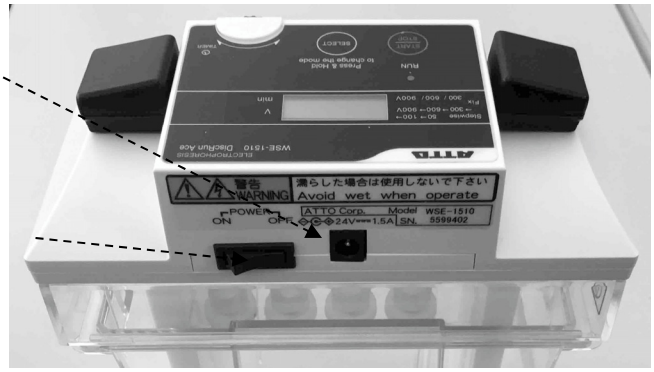
Electrode connectors

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

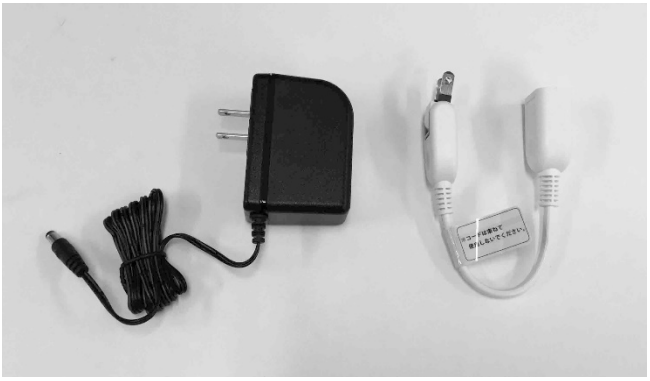
AC adapter jack

Main switch (Power switch)

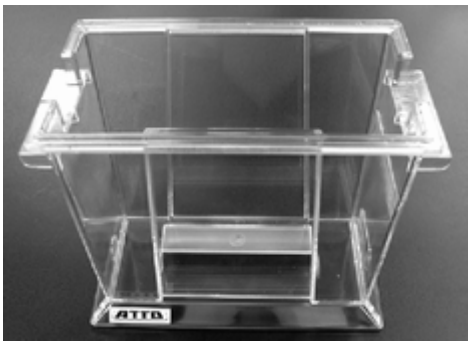


Rear view

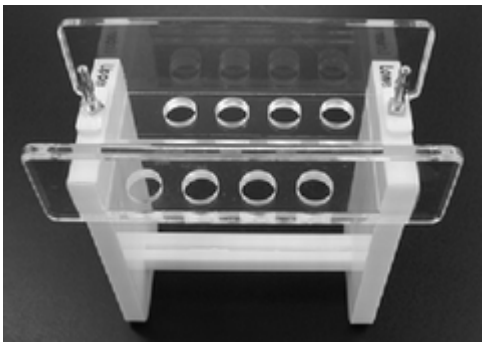
AC adapter



2.1.2 Other parts

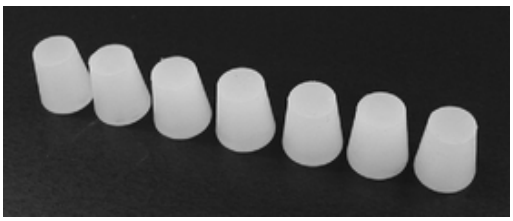


Lower chamber

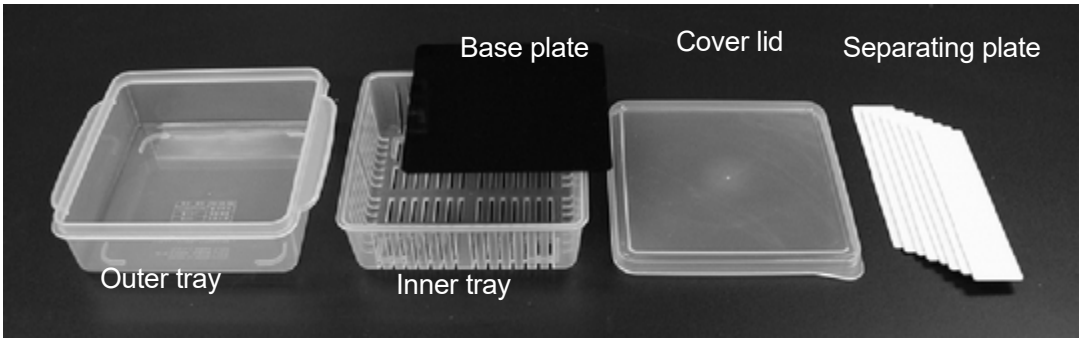


Upper chamber

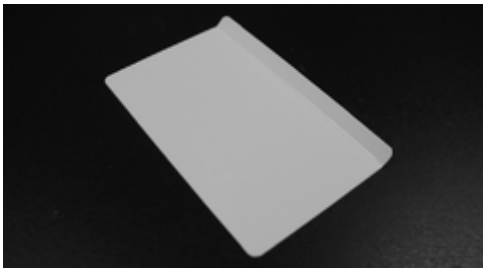
Grummets for EP & IEF Tubes



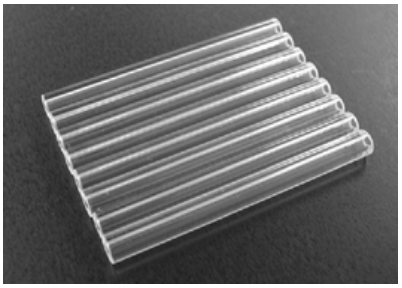
No. 02 Silicone cap



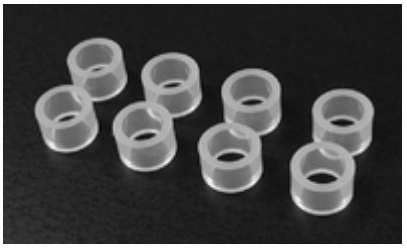
Agar gel tray



Gel carrier

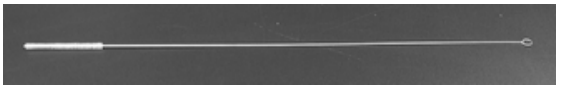


Option: Gel column



Option: Membrane fixing ring (O-ring)

Option: Rod gel casting syringe



Option: Column cleaning brush

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

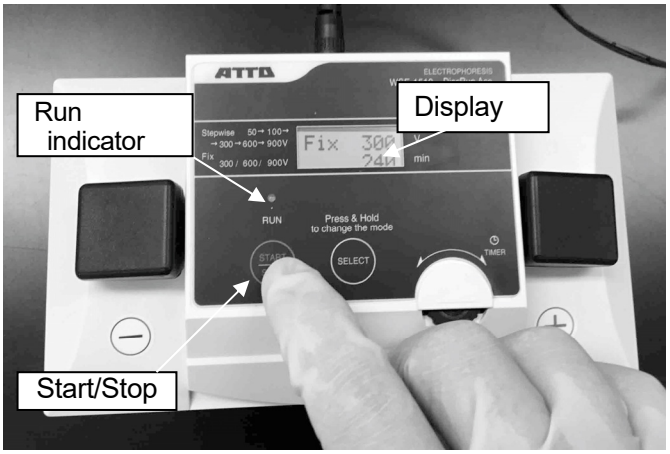
2.1.3. Operation panel

(1) Display

Selected output mode, the remaining time of the energizing setting while running is displayed on a count-down basis.

The output mode is displayed on the top, the time is displayed on the bottom and shown in minutes.

''End'' is displayed at the end of energization, ''Shor Err'' or ''Open Err'' are shown when a short or open error occurs.



Operation panel

(2) Run indicator

This indicator blinks when running electrophoresis. When short open error occurs, it blinks faster. Other than the above, the light is off.

(3) Start/Stop

Press this key to start or stop electrophoresis. The run indicator blinks while running. It stops when pressing the button once again.

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.

(4) Mode Select key

Select output mode. By long pressing of the button, output mode switches to Fix or Step. It will be shown on the display.

For agarose IEF

Mode	Gel size	Setting	Time
Fix	Compact gel size(50mm)	Fix 300V	150min.
	Mini size(75mm)	Fix 300V	240min.
Step	Mini size(75mm)	Step 50V	5min.
		Step 100V	5min.
		Step 300V	5min.
		Step 600V	115min.
		Total	130min.
	Mini size(75mm)	Step 50V	10min.
		Step 100V	10min.
		Step 300V	10min.
		Step 600V	10min.
		Step 900V	70min.
		Total	110min.

For O'Farrell system

Mode	Gel size	Setting	Time
Step	Mini size(75mm)	Step 50V	10min.
		Step 100V	30min.
		Step 300V	900min.
		Step 900V	60min.
		Total	1000min.

(5) Timer dial

Turn the dial clockwise or counter-clockwise to choose appropriate value to set. To set continuous output, select “0.”

Display

2.2. Specifications

Number of simultaneous electrophoresis gels: Max. 8

Quantity of gel tray: Upper electrode solution: 40 mL

Lower electrode solution: 550 mL

Power supply module

Output mode:	Constant voltage
Output voltage:	Constant voltage output (Fix) 300, 600, 900V Step-up output (Step) 50,100,300, 600, 900V
Output current:	12 mA max.
Safety functions:	No-load detection/ short-circuit detection
Timer:	Continuous/ 1 – 999 min/ Remaining time display *Under 0 min. setting; Fix mode: Timer-off / Lapse time display Step mode: Skip output setting of 0 input
Alarm:	When output stops, time is up, an error is detected
Power	13W

Materials

Upper chamber: Acrylic resins

Lower chamber: Polycarbonates

Power supply case: ABS

Safety cover: Polycarbonates

AC adapter:

Input:	100 – 240 VAC, 50/60 Hz
Output:	24 VDC, 1.5A*

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

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	4 hours
Gel preparation, 2nd dimension	09:35 –	
Gel solidification, 1st dimension	13:35 – 13:40	3 min.
Gel cleaning (1), 1st dimension	13:40 – 13:45	3 min.
Gel cleaning (2), 1st dimension	13:45 – 15:45	2 hours
Gel equalizing, 1st dimension	15:45 – 15:55	10 min.

Gel migration, 1st dimension	15:55 – 16:00	5 min.
SDS-PAGE, 2nd dimension	16:00 – 17:30	90 min.
Gel solidification (CBB dyeing), 2nd dimension	17:35 – 17:55	20 min.
Gel dyeing (CBB dyeing), 2nd dimension	17:55 – 18:35	40 min.
Gel decoloring (CBB dyeing), 2nd dimension	18:35 – 19:35	60 min.
Gel storing and image photographing	19:40 –	

⚠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) Sample buffer
- Urea (of high purity)
- Thiourea (special class)
- Complete™ Mini EDTA-free (protease inhibitor cocktail)
- CHAPS (3-Cholamidopropyl Dimethylammonio Propanesulfonic Acid)
- Triton™ X-100 (polyethyrene glycol mono-p-iso-octylphenyl ether)
- DTT (dithiothreitol)
- Iodoacetamide

(2) For 1st dimension IEF
- Agarose IEF*
- Sorbitol (class 1 or above)
- Urea (of high purity) *
- Thiourea (special class) *
- Pharmalyte™ (cytiva) *
  Code No.: pH 2.5 – 5, pH 4 – 6.5
  pH 5 – 8, pH 8 – 10.5 etc.
  Select the type of Pharmalyte to be used according to the purpose.
  *not required when using agarGEL precast gel
- 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.

(3) 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.

(4) 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 in distilled water, adjust the pH 8.8 to 9.0 with HCl and make a 10 mL solution.

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

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 solution (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 solution (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) 30% Acrylamide Solution (Storable at 4°C for 1 month. Not needed when precast gels are used.)

		Final concentration
Acrylamide	29 g	(29%)
Bis	1.0 g	(1%)

Dissolve in distilled water to make a 100 mL solution.

(8) Separating Gel Buffer (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 to pH 8.8 with HCl and make a 500 mL solution.

(9) Stacking Gel Buffer (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 to pH 6.8 with HCl and make a 100 mL solution.

(10) 10% Ammonium Persulfate (Storable at 4°C for 1 week. Not needed when precast gels are used.)

		Final concentration
Ammonium persulfate	100 mg	(10%)

Dissolve in 1.0 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) SDS equilibration buffer (Storable at 4°C for 2 months.)

		Final concentration
Stacking Gel Buffer	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) 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.

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

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

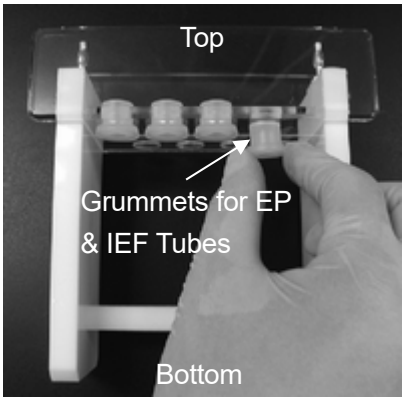
Dissolve in distilled water to make 1,000 mL solution.

5. Gel and Sample Preparation

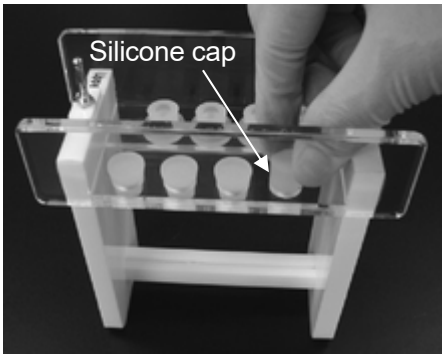
5.1 1st dimension IEF gels

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

Put Grummets for EP & IEF Tubess in the upper chamber from bottom to top. Push the gasket until it stops.

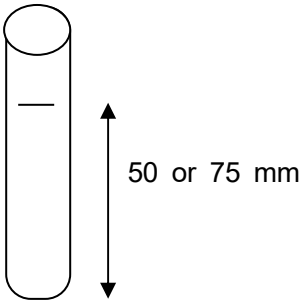


Plug the unused holes of the upper chamber with silicone caps. Push down till it stops.



♦ 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 distilled water for 1 minute or more.

Place the membrane on the end of the columns further from the gel preparation marker and fix it with an membrane fixing ring (O-ring).

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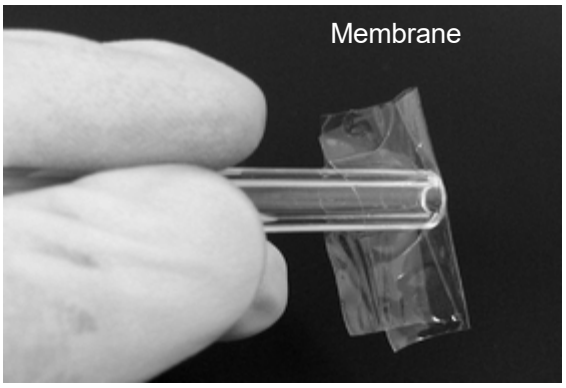
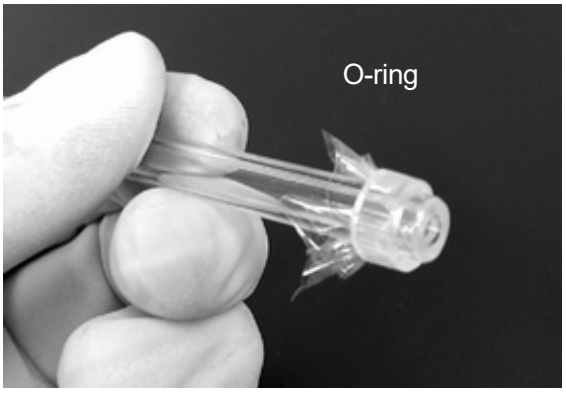
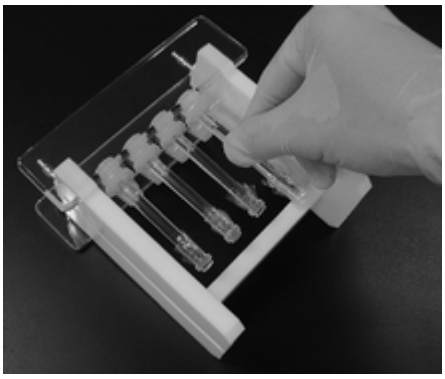


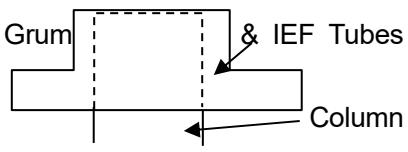


Set the columns on a gel preparation rack (upper chamber).

Insert the column from bottom of the chamber.



Adjust the top of the gel column with the top of the Grummets for EP & IEF Tubes. (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 mini size 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 954 μL.

E.g.) For pH 5 – 10

pH 5 – 8	477 μL	
pH 8 – 10.5	477 μL	Total: 954 μL

E.g.) For pH 5 – 8

pH 5 – 8	954 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 B1.

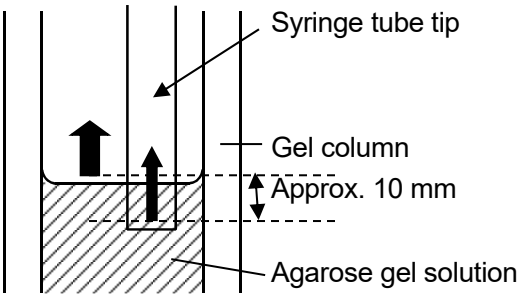
Note: Agarose will be fixed if Reagent B1 is not added quickly.

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.

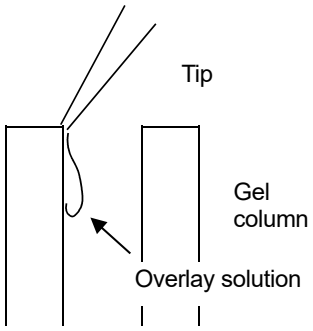
Pour the agarose gel solution into the gel casting syringe, and inject the agarose gel solution into the columns.

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: Air bubbles may clog the gel column if the overlay 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 overnight.

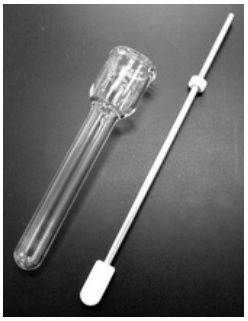
Note: The rod gels can be stored at 5 – 10 °C for up to 5 months. Do not use the gels if they become dry.

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 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.

Restoration of disulfide bounds causes erroneous electrophoresis results: homooligomers and

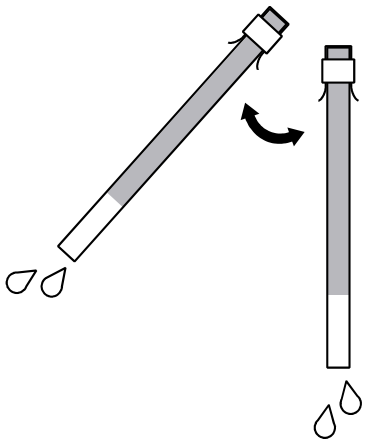
heterooligomers can form and marked vertical and horizontal streaking can appear on 2-D patterns.

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.

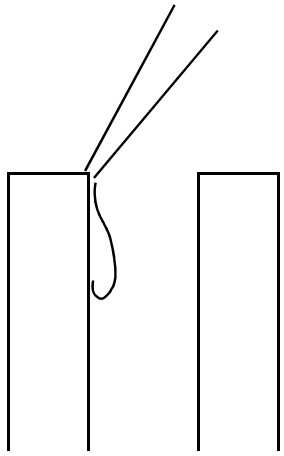


Set the columns on the upper chamber.
Plug the unused holes of the upper chamber with silicone caps.

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

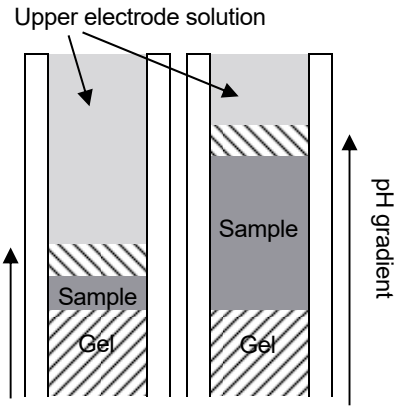
Set the upper chamber into the lower chamber. Apply an appropriate amount of sample solution to the upper end of the first-dimension agarose gel.

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.



Air bubbles may clog the gel column if the upper electrode 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.

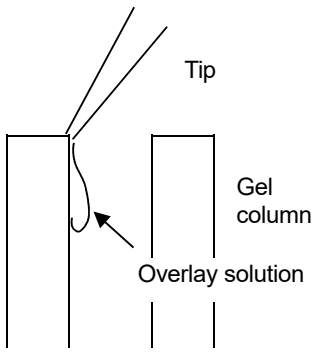


Large sample volume

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.

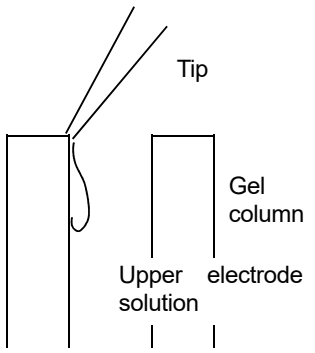
Place the upper electrode solution on the overlay solution up to the top of the gel column. Use a 100μL or finer scale chip and slowly place the solution by sliding it down the inside wall of the gel column.

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.



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

Pour 35mL upper electrode solution into the upper chamber. Gently pour so that the electrode solution will not directly hit the top of the gel column.

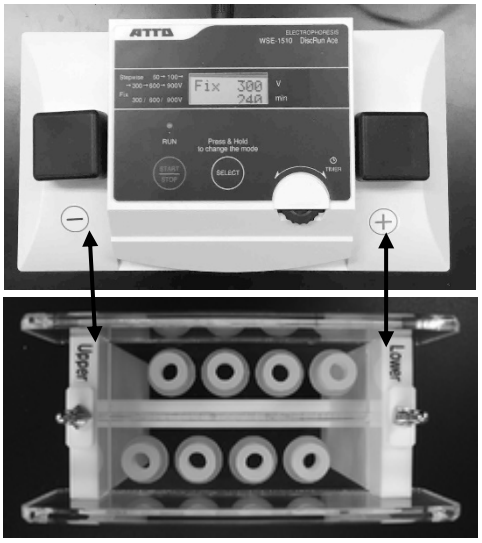


Air bubbles may clog the gel column if the upper electrode solution is poured too quickly.

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

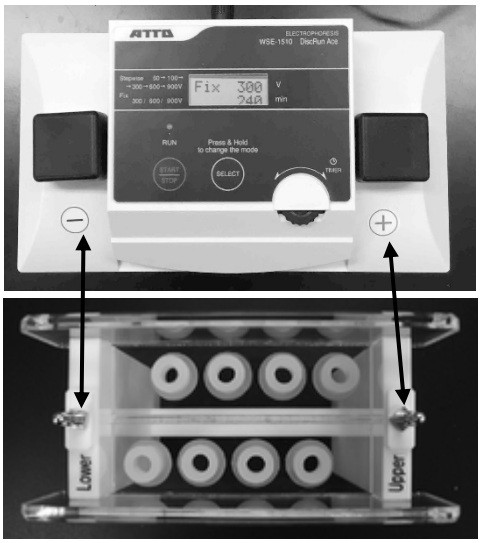
Place the power supply module on the chamber by checking the directionality. The “+” electrode connector of the power supply should match the LOWER electrode terminal on the upper chamber and the “-” connector match the UPPER terminal. Push the “+” and “-” electrode connectors downward.

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



“–“ for “Upper” “+” for “Lower”

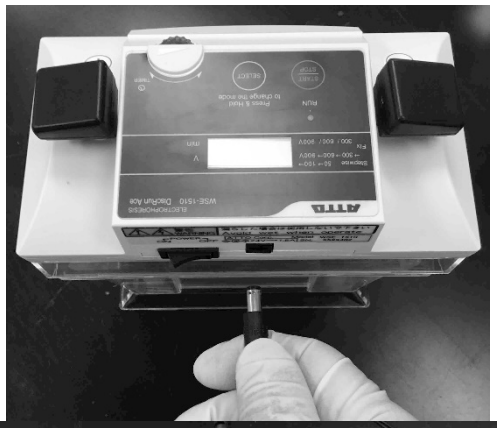
Application from the acid side is possible by reversing the connection. When using this, replace the upper and lower electrode solutions with each other.



“–“ 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.



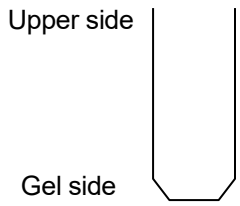
Press Mode Select key to set “Fix, 300V.” Turn the timer dial to set running time. Set 150 minutes for compact size (50 mm) gels or 240 minutes for mini size (75 mm) gels.
After setting the running time, press Start/Stop button to start running.

Immediately after the start of electrophoresis, and as the high-molecular-weight proteins start to migrate, the pores 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 240 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 cut-out using the optional flat comb for mini 2-D and flat comb for compact 2-D.



Cross-sectional view of 2-D flat comb

Gel

Gel Concentration:

Gel Concentration (%T)	Molecular Separation Range
5%	80-400 kD
7.5%	40-200 kD
10%	20-130 kD
12.5%	14-80 kD
15%	10-60 kD

Decide an appropriate gel concentration. In native PAGE, mobility of molecules is largely dependent on the charge. So, gel concentrations cannot be decided by molecular weights, and they should be decided experimentally.

Composition of Gel Solution:

Volume for 2 gels (in mL)	Separating Gel						Stacking
	5%	7.5%	10%	12.5%	15%	20%	4.5%
30% Acrylamide Solution	3	4.5	6	7.5	9	12	0.9
Separating Gel Buffer (1.5 M Tris-HCl, pH 8.8)	4.5	4.5	4.5	4.5	4.5	4.5	-
Stacking Gel Buffer (0.5 M Tris-HCl, pH 6.8)	-	-	-	-	-	-	1.5
10% Ammonium Persulfate	0.08	0.08	0.08	0.08	0.06	0.06	0.02
TEMED	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water	10.5	9	7.5	6	4.5	1.5	3.6

To make Gel Solution:

Mix the 30% Acrylamide Solution, the Separating Gel Buffer (1.5 M Tris-HCl, pH 8.8) for separating gels or the Stacking Gel Buffer (0.5 M Tris-HCl, pH 6.8) for stacking gels, and the Water.

Soon prior to introducing the gel solution, add the 10% Ammonium Persulfate and the TEMED, and gently and thoroughly mix. When the solution has been uniformly mixed, introduce it into gel casts.

Note: Since the solution with SDS easily raise air bubbles, it should not be stirred intensely.

Note: TEMED initiates polymerization of acrylamide, the solution added with TEMED should be introduced in gel casts immediately.

Separating gel with water overlay would be polymerized in about 40-60 minutes, and stacking gel would be polymerized in about 30 minutes.

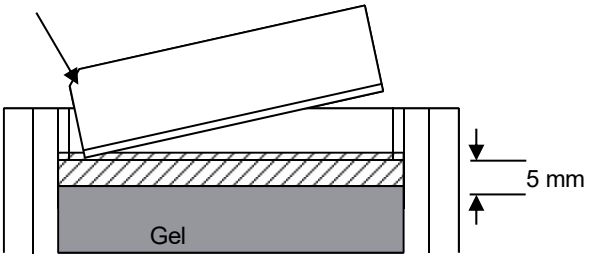
Note: Variation in room temperature affects gel polymerization. To provide better reproducibility of separation, it is recommended to polymerize gel at a given, constant temperature, and temperatures less than 20°C should be avoided.

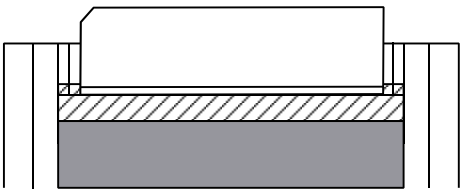
Resolving gels are prepared up to approximately 5mm below the cutout on the gel plate. Inject the stacking gel solution to fill the cutout completely. Insert the 2-D comb obliquely into the cutout on the electrophoretic plate, making sure that the side with the cut comb tip contacts the gel as shown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

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.

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

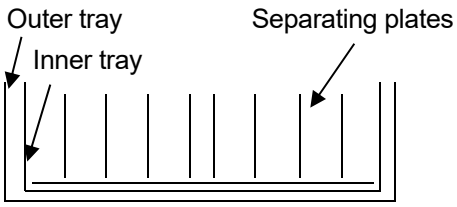




(5) Fixing, Cleaning, and Equilibrium of 1-D Gel

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

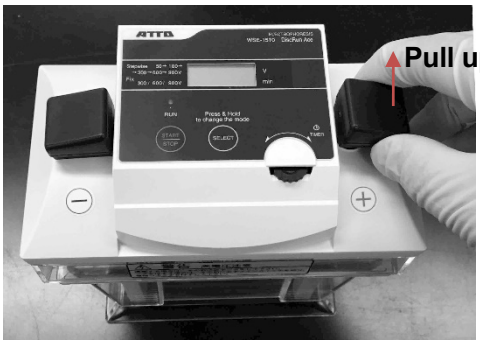
* The 1-D gel solidification solution is strongly acidic. Handle it carefully.



Aga gel tray as assembled

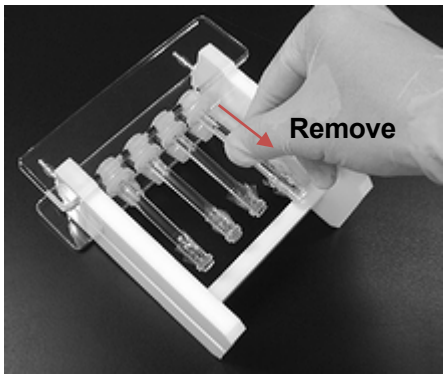
When the 1-D isoelectric electrophoresis has been completed, turn off the main switch on the power supply module and disconnect the AC adapter power plug from the outlet.

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

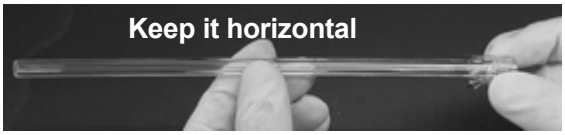


Remove the upper chamber from the lower chamber. Dispose the upper electrode solution. Wash off residual upper and lower electrode solutions from the upper chamber with distilled water.

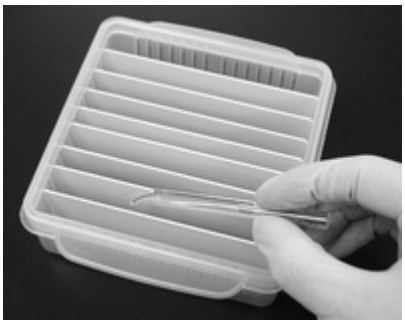
Remove the gel column from the packings.



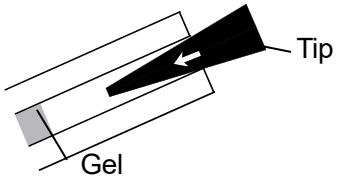
Hold and keep the gel column horizontal and remove the dialysis membrane fixing ring and the dialysis membrane.



On the Aga 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.



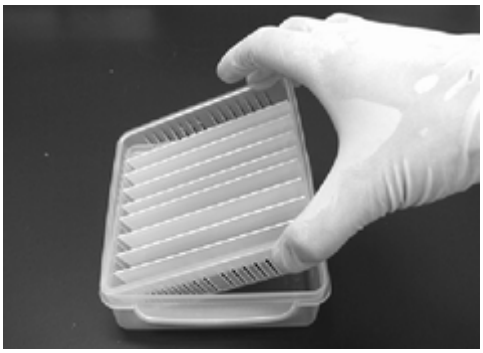
When the gel will not slide off from the column even though you tilt the column, attach a Pasteur pipette rubber cap on the other end of the column and push the gel off from the column.
<u>Gels are soft and easily damaged. Do not give strong shocks to them in the steps described below.</u>



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.

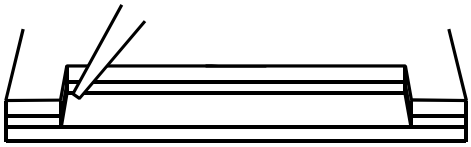
Change the water once again and gently stir for 2 hours.
The gels can be stored 1 to 2 days in the distilled water. If longer storage is necessary, remove the gels from the water, cover them using plastic wrap, and freeze them. The frozen gels can be stored up to 3 month. When using the frozen gels,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

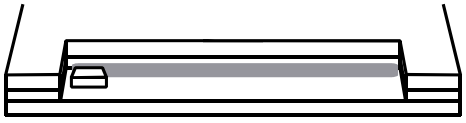
While stirring the gels, soak the molecular weight marker using a square piece of clean filter paper (3 x 3 mm). The guideline of protein mass per band for CBB staining is 200 ng for mini-size system and 100 ng for compact-size system. (1/50 to 1/100 the volume for silver staining.) For 1 mm in thickness 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

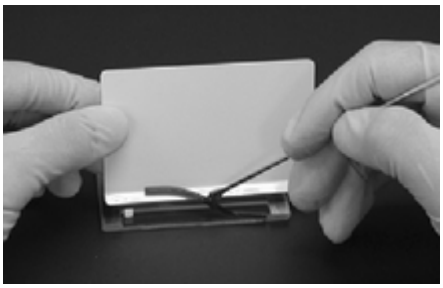
Place the notch of the gel plate upward and forward.



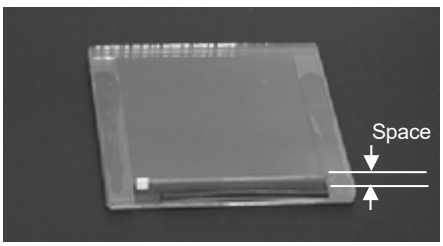
Place a filter paper soaked in the molecular weight marker using forceps.



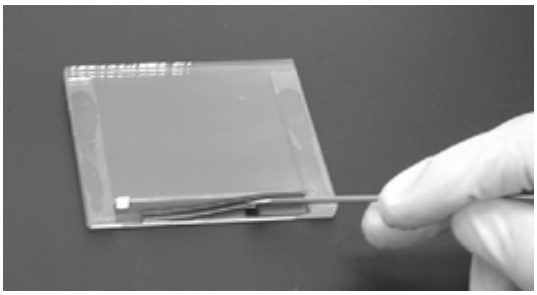
Use gel carrier to place the 1st dimension gel in front of the notch of the plate without damaging the gel.

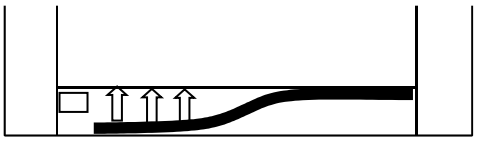


Do not touch the 1st dimension gel to the top of 2nd dimension gel.

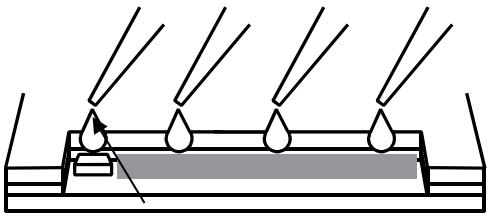


Then carefully adhere the first dimension gel without creating air bubbles.





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.

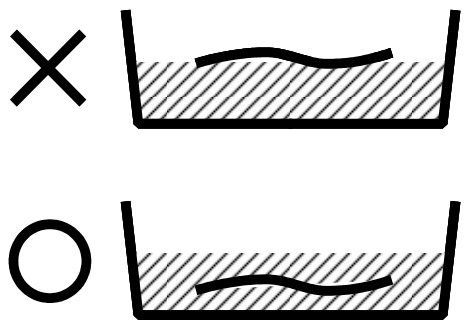


Agarose solution to adhere the 1st dimension gel

Start second dimension SDS-PAGE by setting the vertical mini slab gel used for the second dimension SDS-PAGE or compact gel to the Electrophoresis chamber.
When pouring SDS-PAGE electrophoresis buffer into the upper chamber, be careful that the buffer does not get direct contact with the 1D agarose gel.
When running under constant current conditions of 20mA / gel, energize about 80-90 minutes for mini gels, and 35 minutes for compact gels.
When running under constant voltage of 300V, energize 30-35 minutes for mini gels and about 10-15 minutes for compact gels.
Please run 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/ options/ consumables

2321780	WSE-1510 Disc Run Ace 1 set	
2321659	AC adapter (100-240V/24V 1.5A) 1pc	
2394112	Upper chamber for Disc Run	
2393715	Lower Chamber, MP	Base unit
2392110	Grummets for EP & IEF Tubes 12/pk	Common for electrophoresis & IEF tubes
2392265	No.02 Silicone cap (6pcs) 1 set	
2394180	Mini size gel casting kit for 1-D	
2394181	Compact size gel casting kit for 1-D	
2394250	Aga gel tray	Incl. plates
2394140	Gel carrier for WSE-1510	
2394160	2-D Combs 2/pk, MP	For 2nd dimensional mini gel
2394065	2-D Combs 2/pk, CP	For 2nd dimensional compact gel

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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