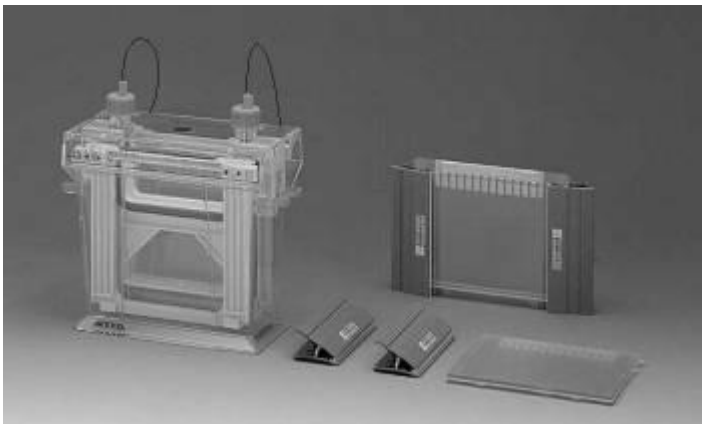
Operating Instructions

AE-6530 mPAGE



Safety Warning

- Prior to using this apparatus, operating and/or handling instructions of this apparatus and apparatus, reagents and/or chemicals that are used with this apparatus must have been understood.
- Use of this apparatus includes application of electricity, which may be fatal or injurious to human. Care should be taken against electricity when applying it to this apparatus.
- Use of this apparatus includes handling of reagents and/or chemicals which may be chemically hazardous or carcinogenic. Care should be taken for protection from hazardous or carcinogenic reagents and/or chemicals when handling them.
- Environments of high temperature, high humidity, dust, corrosive gas, excessive source-voltage fluctuation, excessive electrical noise, and physical shock or vibration should be avoided.
- This apparatus and apparatus that are used with this apparatus should be installed on a flat which is level, solid and stable.
- Prior to operating this apparatus, the apparatus and its components and/or parts should be thoroughly clean and dry. Caution should be exercised against water or moisture which may cause electrical hazards

Contents

Request to first-time users from Atto:

1. When unpacking this set of apparatus, please read “Section 1. Set Composition”, to check and identify the set components.

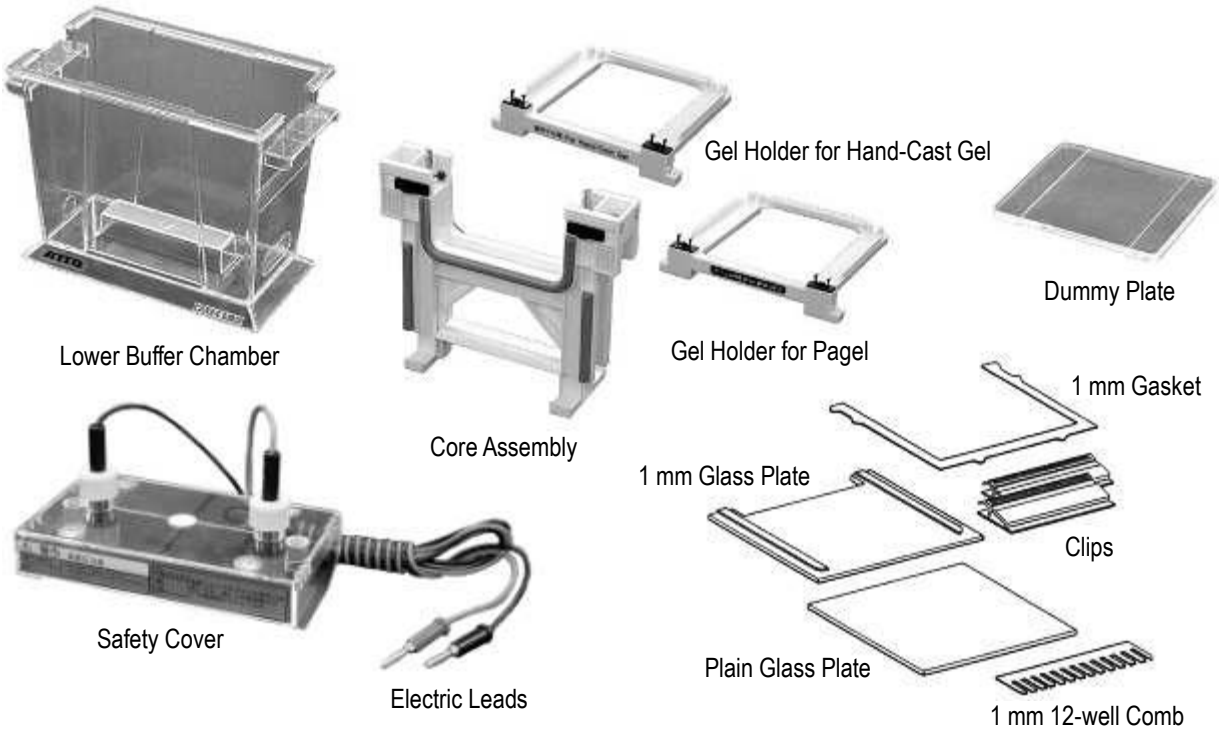
2. First of all prior to operating this apparatus, please read “Section 3. Pre-operation Electric Leads”, attach the electric leads, and practice connection and disconnection of the leads, for your safety against electricity.

3. Prior to operation, please read “Section 4. Pre-operation – Gel Holders & Core Assembly”, and practice methods to set and take out gel-glass plate sandwich. It is essential for operation of this apparatus. After knowing the methods, there will be no difficulty in handling of this apparatus.

4. Prior to carrying out electrophoresis, please go through “Section 5. Operation” practically. The section describes common methods and skills to make the best use of this apparatus.

5. “Section 6. Application – Protein Electrophoresis” and “Section 7. Application – Nucleic Acid Electrophoresis” describe typical protocols offered by Atto. Although they may be different from your own protocols, they have been proven by our long experience. We would be pleased if you would try them or take them into your consideration.

Section 1. Set Composition



A standard set of #2321915 AE-6530 mPAGE or #2321905 mPAGE Chamber, PG is composed of the following components.

	Common Components:		
1	Electrophoresis Chamber (Lower Buffer Chamber)	1	piece
2	Safety Cover	1	piece
3	Electric Leads (each with male & female plugs and a retainer)	1	pair
4	Core Assembly (set in electrophoresis chamber, when shipped)	1	piece
5	Gel Holder for hand-cast gel (set on core assembly, when shipped) Gel Holder for "Pagel" precast gel (set on core assembly, when shipped)	2	pieces
6	7 mm Dummy Plate (only for #2321915 mPAGE) 5 mm Dummy Plate (only for #2321905 mPAGE Chamber, PG)	1	piece
	Components for #2321915 mPAGE only:		
7	1 mm Glass Plate (with 1 mm side-spacers and a bevel notch)	2	pieces
8	Plain Glass Plate (with no side-spacers and no notch)	2	pieces
9	1 mm Gasket (opaque and U-shaped)	2	pieces
10	1 mm 12-well Comb	2	pieces
11	Clip (green colored)	4	pieces

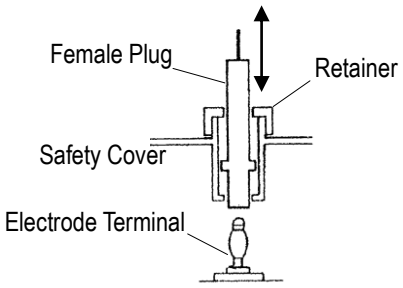
Section 2. Specifications

Application	Polyacrylamide gel electrophoresis of protein and nucleic acid
Gel size	90 mm (width) × 80 mm (length), for hand-cast gel 90 mm (width) × 73 mm (length), for *Pagel* precast gel
Gel thickness	1 mm (standard) or 0.75 m (optional)
Plate size	120 × 102 × 7 mm, for 1 mm thick hand-cast gel 120 × 102 × 6.75 mm, for 0.75 mm hand-cast gel 120 × 100 × 5 mm, for *Pagel* precast gel
Sample well	12 wells per comb (standard) or optional wells
Gel accommodation	Either 1 or 2 gels
Buffer volume	80 + 420 mL, approx.
Material	Polycarbonate and glass, except: platinum for electrodes, silicone rubber for gaskets, polyethylene for standard combs, acrylic for dummy plate, stainless steel for clips
Dimensions (W×D×H)	16.4 × 9.4 × 15.4 cm, approx.
Net weight	0.6 kg, approx.

Section 3. Pre-operation – Electric Leads

Note: Prior to electrophoresis operation, the electric leads must be fixed on the safety cover, and connection and disconnection of the electric leads must be practically understood.

Male Plugs



(Connecting & Disconnecting Electric Lead)

Caution: When shipped, the electric leads (current input leads) are detached from the safety cover. Prior to use, the electric leads must be fixed to the safety cover, so that electricity cannot be applied unless the electrophoresis chamber have been covered by the safety cover, for operator safety.

The electrophoresis chamber (the lower buffer chamber set with the core assembly) has 2 electrode terminals. For running electrophoresis, 1 pair of electric leads are connected to the electrode terminals respectively. The safety cover has 1 pair of vertical, cylindrical openings, where 1 pair of power input leads will be fixed respectively. Each of the electric leads has a male plug at its end, to connect to an electric output terminal of a power supply, and a female plug at another end, to connect to an electrode terminal of the electrophoresis chamber. It also has a retainer which is threaded with the lead and movable through the lead.

Note: The electrophoresis chamber is designed so that upper buffer is cathodic (-) and lower buffer is anodic (+), whichever electric leads are connected to whichever electrode terminals of the chamber.

To Fix Electric Leads:
Insert the female plugs of the electric leads into the cylindrical openings of the safety cover respectively, taking care to match the polarities of the plugs and openings. Fit the retainers of the leads over the cylindrical openings respectively. Ensure that now the plugs can move in the openings with a range but they cannot be removed from the openings. Note that the female plugs are intentionally loose fit so that the plugs can be smoothly connected to and disconnected from the male electrode terminals of the chamber.

To connect the input leads:
Gently push down each female plug of the leads, with fingers, until it stops.

To disconnect the input leads:
While holding the safety cover with a hand, gently pull up each female plug with fingers.

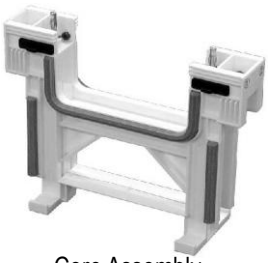
Caution: Do not disconnect the female plugs forcibly by pulling up the safety cover, which may damage the plugs and/or the electrode terminals.

Section 4. Pre-operation – Gel Holders & Core Assembly

Note: Prior to electrophoresis operation, operation of the gel holders and the core assembly to set gels in the electrophoresis chamber and to take out the gels must be practically understood.

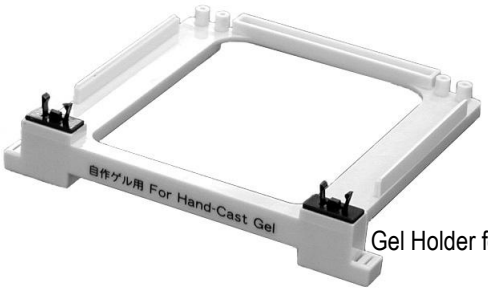
Core Assembly:

The core assembly, either with or without the gel holders, can be freely placed in the lower buffer chamber and taken out of the chamber, by lowering it and lifting it along the slots inside the chamber by hands.

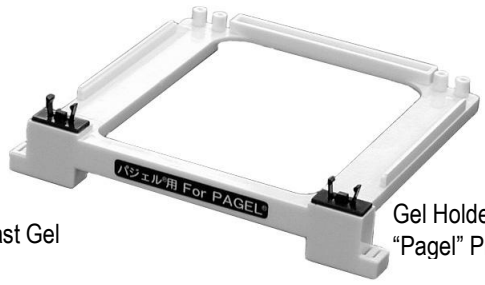


Core Assembly

Gel Holders:



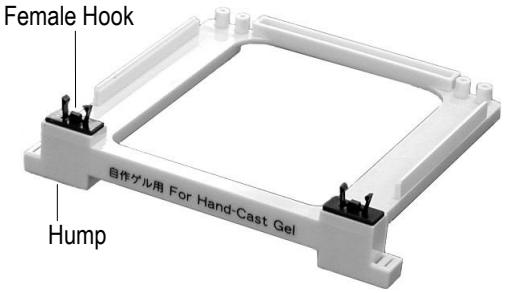
Gel Holder for Hand-Cast Gel

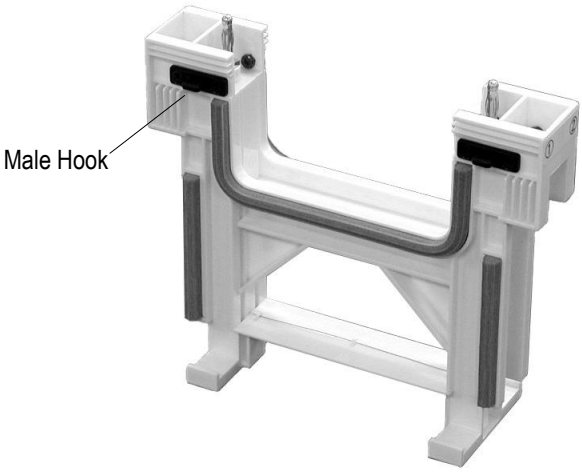


Gel Holder for
"Pagel" Precast Gel

There are 2 types of gel holders, while the core assembly is common. One, labeled "For Hand-Cast Gel", is for a 120 × 102 × 7 mm or 120 × 102 × 6.75 mm gel-plate sandwich of 1 or 0.75 mm thick hand-cast gel. Another, labeled "For Pagel", is for a 120 × 100 × 5 mm gel-plate cassette of 1 mm thick Pagel precast gel.

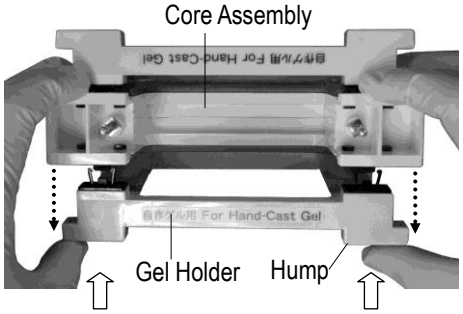
Snap Mechanism:





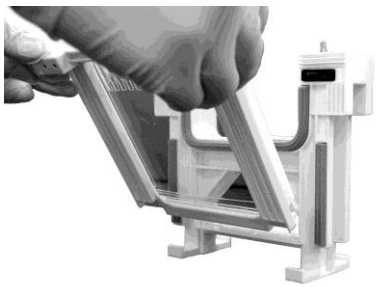
The gel holder is attached to the core assembly and detached from the assembly, by a snap mechanism. The gel holder has a pair of female hooks and a pair of humps. The core assembly has a pair of male hooks on each of its front and back. Pressing the female hooks against the male hooks makes them clasp with each other. Pressing the female hooks against the male hooks, when they are clasping with each other, makes them unclasp. Operation to press the female hooks is made by pushing the humps of the gel holder against the core assembly.

To Detach Gel Holders:

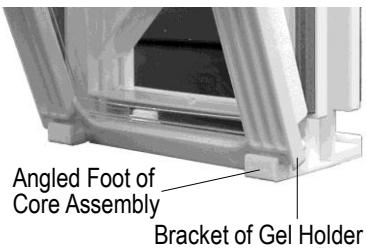


To detach 2 gel holders which have been attached to the core assembly, the gel holders must be detached one by one. To detach a gel holder, either one, push the humps of the gel holder with thumbs, while holding the core assembly with other fingers, not pushing the humps of the both gel holders. Then, detach another gel holder, by pushing the humps with thumbs, while holding the core assembly with other fingers.

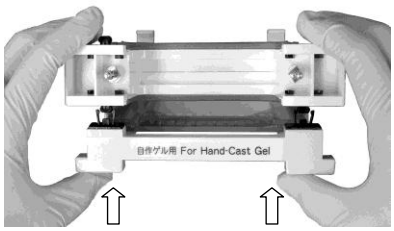
To Attach Gel Holders:



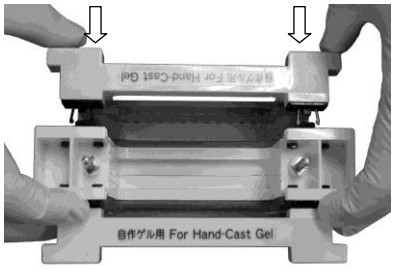
The gel holders must be attached one by one.



Note that the gel holder has 2 brackets at its left and right ends of the bottom and that the core assembly has 2 angled feet at its left and right ends of its bottom, each at front and back.



Match the inside of the brackets of a gel holder with the outside of the angled feet of the core assembly. Push the humps of the gel holder with thumbs, while holding the core assembly with other fingers, until click is felt.



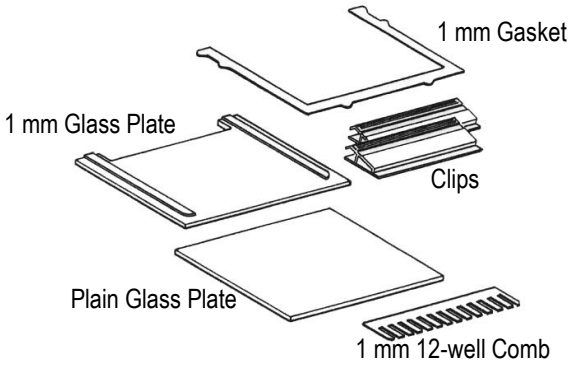
Match the inside of the brackets of another gel holder with the outside of the angled feet of the core assembly. Push the humps of the gel holder with thumbs, while holding the core assembly with other fingers, until click is felt. Do not push the humps of the both gel holders. Pushing the humps of the both gel holders makes one of the gel holders attached and another gel holder detached.

Section 5. Operation

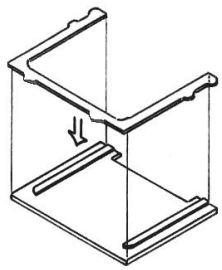
Note: This section describes operation for protein electrophoresis. Operation for nucleic acid electrophoresis is partly different from that for protein electrophoresis, although it is mostly common. Operation for nucleic acid electrophoresis should be adjusted, referring to Section 7 Application – Nucleic Acid Electrophoresis.

5-1 Assembling Gel Cast

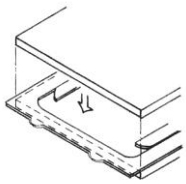
Gel Cast Components:



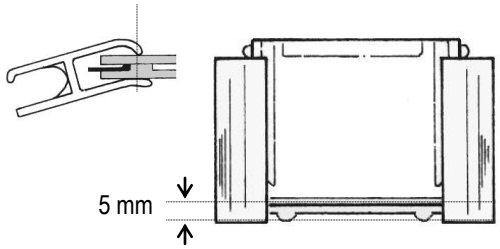
Ensure cleanness of the gel casting components. Especially stain on the glass plates tends to cause air bubbles in gel solution.



Lay the 1 mm glass plate, with its spacers upside, on a clean flat. Lay the 1 mm gasket, with its convex side upside, on the glass plate. Align the top of the gasket with the top of the glass plate, and align the inner edges of the gasket with the outer edges of the side-spacers of the glass plate.



Overlay the plain glass plates, to align the edges of the glass plate with the edges of the 1 mm glass plate, taking care not to move the 1 mm gasket.

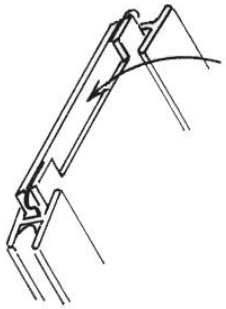


Clamp the glass plates with the pair of clips, so that each clip compress approximately sideways center of the side-spacer of the 1 mm glass plate and the bottoms of the clips are about 5 mm away from the bottoms of the glass plates. Stand the clamped glass plate sandwich vertically on a level flat, and ensure that it is approximately level.

It is recommended to put a level mark on the 1 mm glass plate, in order to secure separation reproducibility. Introducing separation gel solution to a fixed level provides better reproducibility. Once insert the comb into the glass plate sandwich, put a level mark at the level which is about 5 to 6 mm lower than the tooth ends of the comb, and remove the comb.

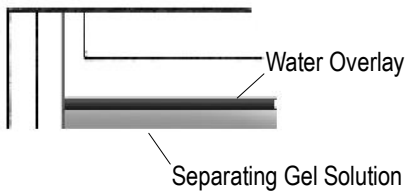
Note: Variation in boundary between stacking gel (concentrating gel) and separating gel tends to vary migration time and/or band figures.

5-2 **Casting Gels**



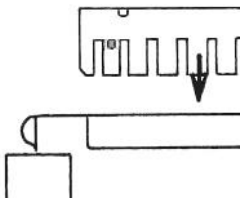
For ease of introducing gel solution, slightly tilt the glass plate sandwich, with the notch of the 1 mm glass plate upside. Gently introduce separating gel solution into the sandwich, taking care not trap air bubbles .

Note: Air bubbles hinder polymerization of gel around the bubbles.

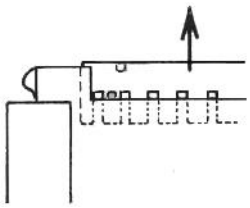


Stand the glass plate sandwich. Gently overlay water to the depth of about 2 to 3 mm. Allow 40 to 60 minutes for separating gel to polymerize.

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.



After polymerization of separating gel, discard the overlay water by tilting the glass plate sandwich. Gently introduce stacking gel solution to the level about 2 mm lower than the notch of the 1 mm glass plate, taking care not to trap air bubbles. Note that the 1 mm 12-well comb has 2 stoppers (small round projections) on one of its surface. Insert the comb between the glass plates, taking care not to trap air bubbles at its teeth, until the stoppers get to the notch of the 1 mm glass plate and thus insertion is stopped. Allow about 30 minutes for stacking gel to polymerize.



After polymerization of stacking gel, gently remove the comb by slowly pushing it up. Rinse the sample wells with a small volume of running buffer, and discard the buffer.

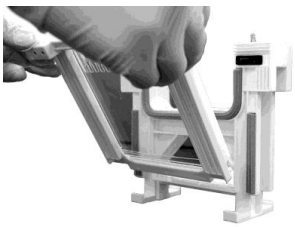
Note:
Unpolymerized gel solution may polymerize after removing the comb, and it may obstruct forming of clear-shaped sample wells.

Gently remove the clips. Gently remove the gasket by slowly pulling it outside. Check up cleanness of surface of the glass plates. Wipe off gel scum and salt precipitate, if any.

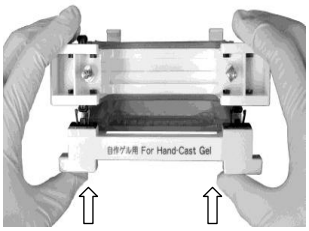
Note: Stain on the glass plates may cause leak of upper running buffer, when the gel-glass plate sandwich has been set in the electrophoresis chamber.

5-3 **Attaching Gels**

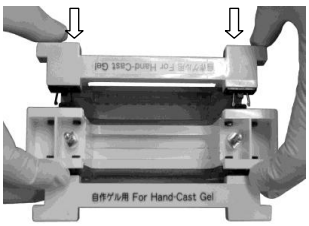
Prepare 2 gel holders, gel holders for hand-cast gel or gel holders for Pagel precast gel. Note that a gel holder has angled edges on its left, right and bottom ends to position a gel-glass plate sandwich of a Pagel precast gel (hereafter called “a gel sandwich”). Place a gel sandwich on the gel holder so that its plain surface will face the gel holder and its notched surface will face outside.



Hold the gel holder so that its surface holding the gel sandwich will face the core assembly, and match the brackets of the gel holder with the angled feet of the core assembly.



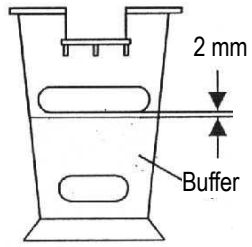
Push the humps of the gel holder with thumbs, while holding the core assembly with other fingers, until the gel holder is clamped with click.



Place another gel sandwich on another gel holder, with its plain surface facing the gel holder. Hold the gel holder, with its surface with the gel sandwich facing the core assembly, and match the brackets of the gel holder with the angled feet of the core assembly. Push the humps of the gel holder with thumbs while holding the core assembly with other fingers until it is clamped with click.

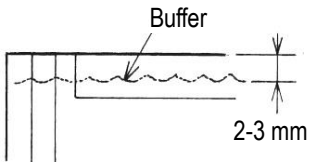
Note: To form an upper buffer trough in the electrophoresis chamber, it is necessary to set 2 gel sandwiches on the core assembly. In case of running only 1 hand-cast gel of either 1 or 0.75 mm thickness, one 7 mm dummy plate should be attached to one of the gel holders, as a substitute for a gel-plate sandwich. In case of running only 1 Pagel precast gel, one 5 mm dummy plate should be attached to one of the gel holders, as a substitute for a precast gel cassette. Note that one of the surfaces of a dummy plate is flat, while the left and right sides of another surface are thickened. When attaching a dummy plate to the gel holder, its surface of which left and right sides are thick should face to the gel holder, i.e. its flat surface will face the core assembly.

5-4 **Setting Gels in Chamber**



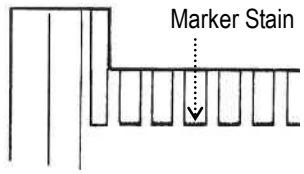
Gently pour about 400 mL of running buffer into the lower buffer chamber, taking care not to raise air bubbles. In the sides of the chamber, there are 2 ovals, higher and lower. When 400 mL of buffer has been introduced, the level of the buffer is about 2 mm lower than the bottom of the higher oval.

Slowly lower the core assembly into the lower buffer chamber, while tilting it in order to avoid trapping air bubbles at its bottom.

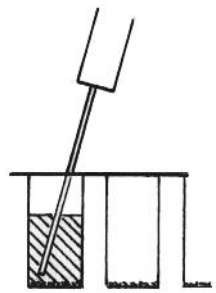


Gently pour about 70 to 80 mL of running buffer in the upper buffer trough so that the level of the buffer is 2 to 3 mm lower than the top of the glass plates.

5-5 **Applying Samples**



Check up sample wells. Remove air bubbles and foreign materials in the wells, if any, and straighten walls of the wells, with the use of a syringe needle or such, taking care not to injure walls and bottoms of the wells. Apply about 2 ③L per well of BPB or such marker stain solution into the sample wells.



Apply samples to the sample wells, with a syringe or micropipet. Sample volume is up to 30 ③L per well in 1 mm thick 12-well gel, but wells of which walls have not been straight formed to give enough height may allow less volumes.

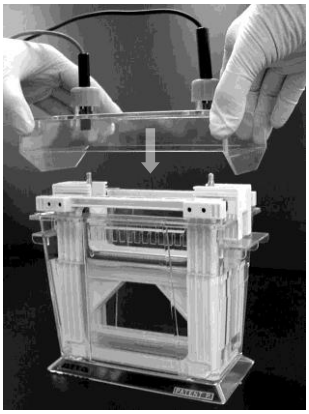
Note:
To provide sharp separation, bring a fine syringe needle or a fine pipet tip as close as possible to the bottom of a sample well, and introduce a sample slowly so that the interface of sample solution will ascend from the bottom. It should be avoided to drop samples into sample wells.

Note: After samples have been applied, a run of electrophoresis should be started instantly.

5-6 **Electrophoresis Run**

Note: After having applied samples, a run of electrophoresis should be started instantly.

Caution: For safety against electricity, all components of the electrophoresis chamber and peripheral equipment which operators may contact should be dry.



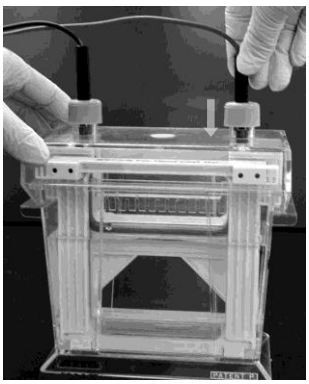
Placing Safety Cover

Prior to connecting to an electrophoresis power supply, ensure that all the surfaces of the electrophoresis chamber, the safety cover and the electric leads and its plugs are thoroughly dry. Check up dryness of the male plugs (metallic banana tip) of the electrode terminals. Wipe off wet and precipitated salt on the plugs.

Prior to connecting the power supply, ensure that mains electricity and output of the electrophoresis power supply are turned off.

Gently place the safety cover on the electrophoresis chamber, with hands.

Note:
It is not to necessary to consider polarity of electricity to be supplied by the power supply, i.e. to consider polarities of the electric leads or polarities of the electrode terminals of the electrophoresis chamber. Regardless of polarity of electricity supplied, upper buffer is cathodic and lower buffer is anodic in the AE-6530 chamber.



Connecting Lead Plug

Gently push down the male plug of either electric lead with fingers until it stops, to connect the plug to the female plug of the corresponding electrode terminal. Gently push down the male plug of another electric lead with fingers until it stops, to connect it to the female plug of another electrode terminal.

Ensure that output of the power supply is turned off, connect the male plugs of the electric leads to the output terminals of the power supply.

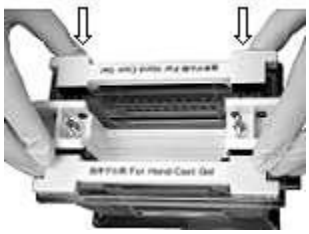
Start a run of electrophoresis by turning on output of the power supply.

A run would be ended when marker stain descends to a level about 5 to 6 mm upper from the bottom of a gel. Stop the run by turning off the output of the power supply.

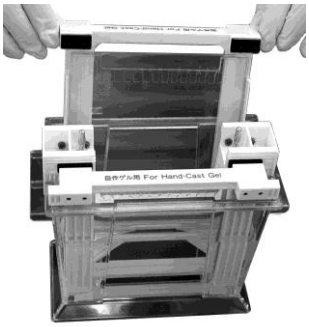
Prior to disconnecting the electric leads, turn off the mains electricity of the power supply. While holding the safety cover with hand, gently pull up the female plug of either electric lead with fingers until it stops, to disconnect the plug. While holding the safety cover with hand, gently pull up the female plug of another electric lead with fingers until it stops, to disconnect the plug.

While holding the electrophoresis chamber with hand, gently pull up the safety cover with hand to remove the cover.

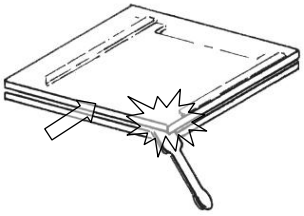
5-7 **Taking Out Gels**



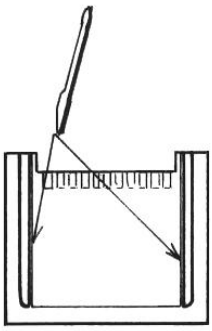
Push the humps of either of the gel holders, while holding the core assembly with other fingers until click is felt, and the gel holder will be detached from the core assembly.



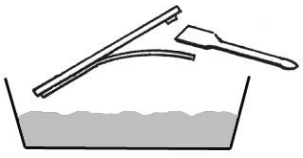
Gently take out the gel holder, detached, with hands. Detach another gel holder, and take out the gel holder. Remove the gel sandwiches from the gel holders.



Lay the gel sandwich on a clean flat with its plain glass plate upside, and lightly lift it with a hand. Gently insert a spatula or such flat blade between the glass plates, nearly at the middle of the bottom of the gel sandwich. Gently pry the glass plates, to peel the glass plates, and usually the plain glass plate will peel off the gel and the gel will remain sticking on the notched glass plate. Corners of the glass plates should not be pried, since prying the corners may break the glass plates.



Wet the blade of a spatula or knife with buffer or water. Gently cut the side edges of the gel along the inner edges of the side-spacers with the spatula, to separate the gel from the side-spacers, taking care not to injure the gel.



Hold the notched glass plate keeping the gel below, over a staining solution in a vessel. Peel off the gel, by gently inserting a wetted spatula or knife between the gel and the glass plate, so that the gel will sink into the solution.

Subject the gel to staining, destaining or such subsequent operation.

5-8 **Cleaning After Use**

Note: The electrophoresis chamber and the other components should be cleaned before buffer and gel residue dry. Dried buffer and gel residue make cleaning difficult.

Note: When cleaning the core assembly, special care should be taken not to damage the platinum electrode wires which are very fragile.

Lower Buffer Chamber, Core Assembly & **Gel Holders:**

Before buffer on them dry, wash them (and a dummy plate if used) with tapping water, and rinse with pure water. Usually it would clean them enough. If stain is persistent, wash down with soft sponge or such. After cleaning, dry them at room temperature.

Safety Cover:

The safety cover should not be washed. If it is stained, clean it with paper towel or such wetted with water or alcohol. Other organic solvents should not be used.

Gel Cast Components:

Before the glass plates, the combs and the U-shaped gaskets dry, clean them with sponge soaked with mild detergent, taking care not to injure them. Special care should be taken against gel residue. Gel residue, once has dried, is difficult to be cleaned off.

The notched glass plates should not be kept immersed in water for a long time, which may possibly cause peeling of the side-spacers on it.

Section 6. Application – Protein Electrophoresis

6-1 Reagents

Note: Reagents should be of electrophoresis grade or equivalent high purity grades.

Acrylamide	Not required for running "Pagel" precast gels
N,N'-methylene-bis-acrylamide (Bis)	Not required for running "Pagel" precast gels
Tris (hydroxymethyl) aminomethane (Tris)	
Sodium dodecyl sulfate (SDS)	For SDS-PAGE only
HCl	
Ammonium persulfate	Not required for running "Pagel" precast gels
N,N,N',N'-tetramethyle-ethylenediamine (TEMED)	Not required for running "Pagel" precast gels
Glycine	
Bromophenol blue (BPB)	
Glycerin	
2-mercaptoethanol	For SDS-PAGE only
Coomassie brilliant blue G-250 or R-250	
Methanol	
Acetic acid	

6-2 Stock Solutions

30% Acrylamide Solution:

Acrylamide	29.2 g
Bis	0.8 g
Dissolve in water	to 100 mL
Store at 4°C	

Separating Gel Buffer (1.5 M Tris-HCl, pH 8.8)**:**

Tris	18.2 g
SDS	0.4 g
Dissolve in water and adjust to pH 8.8 with HCl	
Water	to 100 mL
Store at 4°C	

Stacking Gel Buffer (0.5 M Tris-HCl, pH 6.8)**:**

Tris	6.1 g
SDS	0.4 g
Dissolve in water and adjust to pH 6.8 with HCl	
Water	to 100 mL
Store at 4°C	

10% Ammonium Persulfate:

Prepare when use	
Ammonium persulfate	0.1 g
Water	1 mL
Dissolve	
Storable for 1 week at 4°C	

Sample Treatment Solution:
(as an example)

SDS	0.1 g	1%
2-mercaptoethanol	0.1 mL	1%
Stacking gel buffer (0.5 M Tris-HCl, pH 6.8)	1 mL	50 mM
Glycerin	2 mL	20%
Water	to 10 mL	
Store at 4°C		

Running Buffer:

Tris	1.5 g	25 mM
SDS	0.5 g	0.1%
Glycine	7.2 g	192 mM
Dissolve in water	to 500 mL	
pH adjustment not required		
Store at room temperature		

Marker Stain:

BPB	1 mg
Glycerin	0.1 mL
Water	0.9 mL
Store at 4°C	

CBB Staining Solution:

Coomassie Brilliant Blue	1 g
Methanol	300 mL
Acetic acid	100 mL
Water	600 mL
Filter through filter paper	
Seal and store at room temperature	

CBB Destaining Solution:

Methanol	300 mL
Acetic acid	100 mL
Water	600 mL
Seal and store at room temperature	

6-3 **Sample Preparation**

Note: Methods for sample preparation vary according to samples and purposes of separation. Method for a respective application should be decided, by referring to reference papers or experimentally. The following is a simple example.

Note: For native PAGE, SDS and 2-mercptoethanol are not applied.

Dissolve samples in the Sample Treatment Solution (Section 6-2). Dry samples are dissolved in the solution, to 1 to 2 mg/mL concentration. Samples containing less moisture such as tissues are homogenized after adding the solution. To solution samples, especially dilute samples, reagents, instead of the preparation solution, may be directly added in order to avoid dilution, as an example shown below.

Serum	20 µL
Water	570 µL
10% SDS solution	100 µL
2-mercaptoethanol for SDS PAGE, or Water for native PAGE	10 µL
Stacking gel buffer (0.5 M Tris-HCl, pH 6.8)	100 µL
Glycerol	200 µL

Heat a treated sample contained in a sealed tube, in a bath, gradually from room temperature, and heat at 100°C for 1 to 2 minutes, and take it out from the bath.

If insolubles or impurities exist in treated samples, remove them by centrifugation. Note that insolubles or impurities cause vertical stripes in separated bands.

6-4 **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 Gel
	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 3% 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.

6-5 Electricity

20 mA per gel (40 mA for 2 gels) constant current % 70-90 minutes approx.

Voltage would be about 80 V at the start of run and about 200 V at the end of run.

Note: Electricity is dependent on gel formulation, polymerization (variable due to ammonium persulfate content, TEMED content, and temperature), buffer formulation, sample solution composition, temperature and such conditions. Adjustment should be made according to respective applications.

6-6 Staining

To stain gels with CBB, as an example:

Immerse gels in the CBB Staining Solution for 2 hours or more, taking care to prevent the gels from sticking to the bottom of the vessel. Discard the solution.

Immerse the gels in the CBB Destaining Solution, taking care to prevent the gels from sticking to the bottom of the vessel, and gently shake, while replacing the solution with fresh solution several times, for several hours or overnight, until the gel background becomes transparent.

Section 7. Application – Nucleic Acid Electrophoresis

7-1 Reagents

Note: Reagents should be of electrophoresis grade or equivalent high purity grades.

Acrylamide	Not required for running “Pagel” precast gels
N,N’-methylene-bis-acrylamide (Bis)	Not required for running “Pagel” precast gels
Tris (hydroxymethyl) aminomethane (Tris)	
Boric acid	
EDTA 2Na ($Na_2EDTA\ 2H_2O$)	
Ammonium persulfate	Not required for running “Pagel” precast gels
N,N,N’,N’-tetramethyle-ethylenediamine (TEMED)	Not required for running “Pagel” precast gels
Glycine	
Bromophenol blue (BPB)	
Sucrose	
Ethidium bromide	

7-2 Stock Solutions

30% Acrylamide Solution:

Acrylamide	29.2 g
Bis	1.0 g
Dissolve in water	to 100 mL
Store at 4°C	

5X TBE Buffer:

Tris	53.9 g
Boric acid	27.5 g
EDTA 2Na	3.7 g
Dissolve in water	to 1000 mL
pH adjustment not required	
Store at room temperature	

10% Ammonium Persulfate:

Prepare when use	
Ammonium persulfate	0.1 g
Water	1 mL
Dissolve	
Storable for 1 week at 4°C	

Running Buffer for Hand-Cast Gel (1X TBE Buffer)**:**

5X TBE Buffer	100 mL
Water	to 500 mL
Store at room temperature	

Running Buffer for Pagel Precast Gel:

Tris	1.5 g	25 mM
Glycine	7.2 g	192 mM
Dissolve in water	to 500 mL	
pH adjustment not required		
Store at room temperature		

Marker Stain:

BPB	0.4 g
Sucrose	6.0 g
Water or *TE buffer	to 10 mL
Store at 4°C	

*TE Buffer: 10 mM Tris-HCl pH 8.0, 1 mM EDTA

Ethidium Bromide Stock Solution:

Ethidium bromide	50 mg
Water	to 10 mL
Store in the dark at room temperature	

Ethidium Bromide Staining Solution:

EDTA Staining Solution	% 1
Water	% 1000
Store in the dark at room temperature	

7-3 Sample Preparation

Note: Difference in salt concentration of samples interferes with separation and results in disturbed bands. Especially, samples of high salt concentration, such as restriction enzyme H buffer, affects mobility and band separation of adjacent lanes.

Unify salt concentration of samples as far as possible. Samples of high salt concentration should be once precipitated in ethanol and then dissolved in buffer, in order to have the same concentration as that of other samples.

Add 1/10 volume of the Marker Stain solution to samples, and mix.

7-4 **Gel**

Gel Concentration:

Gel Concentration (%T)	Molecular Separation Range	
	Hand-Cast Gel 1X TBE Buffer	"Pagel" Precast Gel Tris-Glycine Buffer
5%	80-500 bp	250-7000 bp
7.5%		100-2000 bp
8%	60-400 bp	
10%	50-300 bp	50-500 bp
12.5%	40-200 bp	
15%	25-150 bp	

Decide an appropriate gel concentration according to molecular weights of samples.

Composition of Gel Solution:

Volume for 2 gels (in mL)	5%	6%	7.5%	8%	10%	12.5%	15%
3% Acrylamide Solution	3.3	4.0	5.0	5.3	6.7	8.3	10
5X TBE Buffer	4.0	4.0	4.0	4.0	4.0	4.0	4.0
10% Ammonium Persulfate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TEMED	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water	12.6	11.9	10.9	10.6	9.2	7.6	5.9

To make gel solution, mix the 3% Acrylamide Solution, the 5X TBE Buffer, 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: TEMED initiates polymerization of acrylamide, the solution added with TEMED should be introduced in gel casts immediately.

Introduce the gel solution into a gel cast to the level about 2 mm lower than the notch of the notched glass plate.

Insert a comb. Allow about 30 minutes for gel polymerization.

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.

7-5 **Electricity**

60 V constant voltage % 120 minutes approx.

Current would be about 15 mA per 2 gels at the start of run and about 10 mA per 2 gels at the end of run. It is suggested to limit current at 20 mA per 2 gels.

In case of constant current electricity, current should be adjusted according to quantity of gels, i.e. current for 2 gels = current for 1 gel % 2.

Note: Electricity is dependent on gel formulation, polymerization (variable due to ammonium persulfate content, TEMED content, and temperature), buffer formulation, sample solution composition, temperature and such conditions. Adjustment should be made according to respective applications.

7-6 **Staining**

To stain gels with ethidium bromide, as an example:

Immerse gels in the Ethidium Bromide Staining Solution for 20-30 minutes, taking care to prevent the gels from sticking to the bottom of the vessel. Discard the solution and rinse the vessel with water.

Immerse the gels in water, taking care to prevent the gels from sticking to the bottom of the vessel, and gently shake for 10-20 minutes. For gels of higher concentrations, it takes longer time for destaining.

Section 8. Trouble Shooting

Symptom	Cause	Remedy
Gel not polymerized		
	Wrong formulation	
		Check up gel formulation and mix stock solutions again. If not successful, stock solution formulation and prepare new stock solutions.
	Ammonium persulfate aged	
		Prepare ammonium persulfate when using, or store at 4°C within 1 week.
	Low temperature of room or gel solution	
		Polymerize at about 20-40°C
Air bubbles in gel		
	Glass plates or combs stained	
		Clean glass plates and combs soon after use before drying, and store free from dust. Do not touch them with naked hands when operating.
Streaks in gel (may affect band separation)		
	Uneven polymerization velocity Caused from uneven temperature distribution in glass plates, combs or gel solution.	
		Warm glass plates, combs and gel solution (without ammonium persulfate and TEMED) to about 37°C with a thermostatic bath. Outside the bath, pour the gel solution, and add ammonium persulfate and TEMED. May be polymerized in the bath.
Wells not straight rectangular		
	Acrylamide monomer residue polymerized after removing combs	
		Remove combs soon prior to electrophoresis. Rinse wells with water or buffer after removing combs.
Gel solution leaks from plate sandwich		
	U-shaped gasket not aligned or caught by glass space and glass plate	
		Realign U-shaped gasket and reassemble glass plates
	U-shaped gasket or glass plates injured	
		Replace the gaskets or glass plates
	Clips aged and compression effect fallen	
		Replace the clips.
Gel sandwich not sit in gel holder		
	Glass plates sheer with each other	
		Do not slip glass plates when assembling glass plates and clamping with clips, or when taking out Pagel precast gels from a pouch.
Gel holders not sit on core assembly		
	Hand-cast gel or 7 mm dummy plate on gel holder for precast gel	
		Use correct gel holder or correct dummy plate.
Upper buffer leaks from trough		
	Gel holders not correctly attached on core assembly	
		Match brackets of gel holders with angled feet of core assembly, and click both left and right clips.
	Glass plates stained	
		Clean glass plates soon after use before drying. Do not allow gel residue. Wipe off gel residue or dust.
	Level of buffer too high Upper buffer may go through joins of seal gaskets and glass plate by capillary action.	
		Level upper buffer at 2-3 mm lower from the top of glass plates.

Symptom	Cause	Remedy
Upper buffer leaks from upper trough		
	Slight leak in long run	
		1-2 mL of SDS buffer may leak overnight or in 1 day, affecting little.
	Seal gaskets on core assembly damaged or aged	
		Replace or repair the gaskets.
	Pagel or 5 mm dummy plate on gel holders for hand-cast gels	
		Use correct gel holders or dummy plate
Sample does not sink down in well		
	Well stained	
		Blow off dusts or gel pieces with buffer, with micropipet or syringe. Or, remove such with syringe needle.
	Clips aged and compression effect fallen, allowing thin gel on glass walls of well	
		Replace the clips
	Sample gravity light	
		Add enough glycerin or sucrose to sample solution
Removing comb damages walls of well		
	Gel sticks to comb	
		If gel solution is degassed for better polymerization, avoid degassing.
Run-to-run difference in mobility or bands not reproduced		
	Different electricity	
		Apply same electricity. For running 2 gels, current/gel % 2.
	Difference in composition or concentration of buffer or gel	
		Check up composition or concentration
	Difference in pH 1.5 M Tris-HCl buffer pH 8.8 or 0.5 M Tris-HCl buffer pH 6.8 stock solution	
		Check up pH
	Gel aged Even if storable at 4°C, gels gradually and steadily age and decline reproducibility.	
		For reproducibility, use gels on the date being cast, or next day at latest.
	Difference in polymerization	
		It is recommended to avoid the technique to increase ammonium persulfate or TEMED for quickening polymerization but to follow our protocol. Since difference in temperature of room and gel solution affects a little, polymerization with thermostatic bath increases reproducibility.
	Difference in salt concentration of sample solution, room temperature, temperature of running buffer, and other conditions.	
		Keep constant conditions.
Lane-to-lane difference and smiling effect		
	Uneven temperature distribution in gel	
		Follow our protocol to pour enough volume of lower buffer to immerse gel sandwiches in the buffer.
	Running buffer declined buffering effect	
		Avoid repeated use of running buffer, and use fresh buffer.
	Lane-to-lane difference in salt concentration of samples Salt concentration of sample solution affects mobility.	
		Unify salt concentration of sample solutions, by desalting, concentration, dilution (ethanol precipitation for nucleic acid) and such methods. Pay attention to restriction enzyme buffer, diluted system, and etc.

Symptom	Cause	Remedy
Outward diffusion (fan-out spreading) of bands		
	Current leaks sideways from gel to outside of gel-plate sandwich. Clips having declined compression effect allow thin gel between a glass plate and the side-spacers of another glass plate.	
		Replace the clips.
	Current leaks sideways from gel to outside of gel-plate sandwich. Gel slightly or partly peel off glass plates.	
		Handle gel sandwiches carefully not to slide glass plates. Do not use aged gel sandwich, of which gel tends to peel.

Section 9. Maintenance

1. Cleaning

After use, wash the lower buffer chamber and the core assembly with water, and dry at room temperature. To wash the safety cover, remove the electric leads. In case stain is persistent, clean with the use of soft sponge.

Soon after use, clean the glass plates with soft sponge and mild detergent.

Note: Cleaning with acetone and such organic solvents or drying at high temperatures may decolorize or deform the plastic components.

Note: When cleaning the core assembly, care should be taken not to bend or cut the platinum electrode wires.

Note: Use of brush or metallic sponge should be avoided.

Note: The glass plates with side spacers should not be immersed in water for a long time. It may cause peeling of the side spacers.

2. Storage

Store the components in an environment free from direct rays, high temperature, and corrosive gases.

Note: When storing the core assembly, keep all the gaskets on it released from compression, to avoid decrease of their flexibility and seal effect. The gaskets lose their seal effect after long use, and their seal effect should be checked up at times. Attach glass plate sandwiches, with the plain glass plates facing the core assembly, to the core assembly, set the core assembly in the lower chamber, pour water in the upper trough, and observe leak of water.

Note: When storing the U-shaped gaskets, keep them released from compression, to avoid decrease of their flexibility and seal effect.

3. Electric Connectors

Check up the electric leads, the male and female plugs of the electric leads, and the male tips at the electrode terminals of the core assembly. If there are any damage or corrosion, withhold use and repair or replace them.

Section 10. Parts List

Code #	D e s c r i p t i o n	Note
2393010	1 mm Dual Mini Gel Cast	2 pairs of glass plates + 2 gaskets + 4 clips + 2 combs, ready to cast 2 1-mm 12-well gels
2398214	1 mm Plate Set, RM	1 pair of glass plates + 1 gasket, for 1 mm gel
2398230	1 mm Glass Plates 2/pk, RM	Notched plate with 1 mm side-spacers
2398232	Plain Glass Plates 2/pk, RM	Rectangular without notch & side-spacers
2398237	1 mm Gaskets 3/pk, RM	Opaque, U-shaped gasket for 1 mm gel
2398239	Clips 4/pk, RM	2 required for casting 1 gel
2398269	1 mm 12-well Combs 2/pk, RM	
2328375	1 mm 6-well Comb, RM	
2328376	1 mm 8-well Comb, RM	
2328422	1 mm 20-well Comb, RM	
2398255	1 mm 21-well Combs 2/pk, RM	
2399041	1 mm Blank Comb, RM	
2328934	1 mm Preparative Comb, RM	1 sample well + 1 reference well
	1 mm special well comb	Consult
2393012	0.75 mm Dual Mini Gel Cast	2 pairs of glass plates + 2 gaskets + 4 clips + 2 combs, ready to cast 2 0.75-mm 12-well gels
2393014	0.75 mm Plate Set, RM	1 pair of glass plates + 1 gasket, for 0.75 mm gel
2398228	0.75 mm Glass Plates 2/pk, RM	Notched plate with 0.75 mm side-spacers
2398232	Plain Glass Plates 2/pk, RM	Rectangular without notch & side-spacers
2398226	0.75 mm Gaskets 3/pk, RM	Opaque, U-shaped gasket for 0.75 mm gel
2398271	0.75 mm 12-well Combs 2/pk, RM	
2398239	Clips 4/pk, RM	2 required for casting 1 gel
2398247	7 mm Dummy Plate, AE-6530	Plastic plate for running 1 hand-cast gel only
2393074	5 mm Dummy Plate, AE-6530	Plastic plate for running 1 "Pagel" precast gel only
2393726	Gel Holders 2/pk, AE-6530	For hand-cast gels
2393731	Pagel Holders 2/pk, AE-6530	For "Pagel" precast gels
2393608	Green Seal Gasket, 1 m	Foamed silicon rubber gasket
2393710	Core Assembly, AE-6530	Incl. electrodes
2393720	Safety Cover, AE-6530	Incl. power leads
2328441	Safety Leads	Incl. retainers
2393715	Lower Chamber, AE-6530	Base unit

Code #	D e s c r i p t i o n	Note
	Parts for 2-D Electrophoresis:	
2398398	Notched Glass Plate with 9 Spacers	For 6 rod gels
2398395	8-teeth Comb	For 6 rod gels
2398232	Plain Glass Plates 2/pk, RM	Included in standard AE-6400 set
2398237	1 mm Gaskets 3/pk, RM	Included in standard AE-6400 set
2398239	Clips 4/pk, RM	Included in standard AE-6400 set
2398391	Rectangel Parts Set	1 pair of glass plates + 1 gasket + 2 clips + 1 comb, to cast 6 rod gels
2398515	2-D Comb, RM	
2392380	Gel Support Films 50/pk	14 × 26 cm, polypropylene
2321915	AE-6530 mPAGE	
	1 electrophoresis chamber complete with a safety cover with power leads, a core assembly, 2 gel holders (for hand-cast gels) and a 7 mm dummy plate (for running 1 gel only) + 2 pairs of glass plates + 4 clips + 2 clips, ready to cast and run 2 1-mm 12-well gels	
2321900	AE-6530 mPAGE Chamber	
	1 electrophoresis chamber complete with a safety cover with power leads, a core assembly, 2 gel holders (for hand-cast gels) and a 7 mm dummy plate (for running 1 gel only)	
2321905	AE-6530 mPAGE Chamber, PG	
	1 electrophoresis chamber complete with a safety cover with power leads, a core assembly, 2 gel holders (for “Pagel” precast gels) and a 5 mm dummy plate (for running1 precast gel only)	
2321112	AE-6530 mPAGE, RG	
	1 electrophoresis chamber complete with a safety cover with power leads, a core assembly, 2 gel holders (for hand-cast gels) & a 7 mm dummy plate (for running 1 gel only) + 1 pair of glass plates + 2 clips + 1 comb, ready to cast and run 6 rod gels	

Memo:

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 use 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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