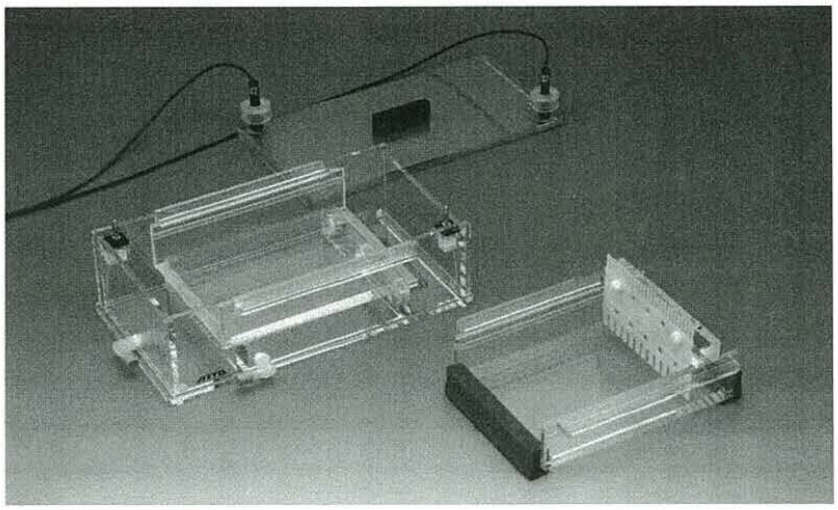
Operating Instructions

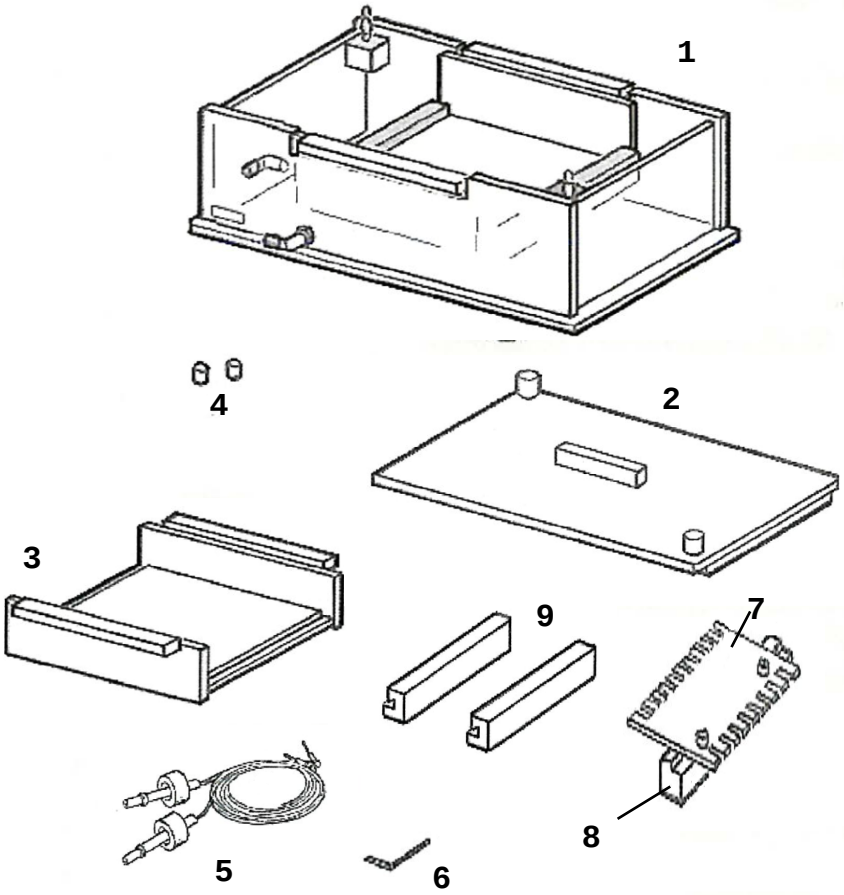
AE-6111 Agarose EP Kit



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

Section 1. Set Composition



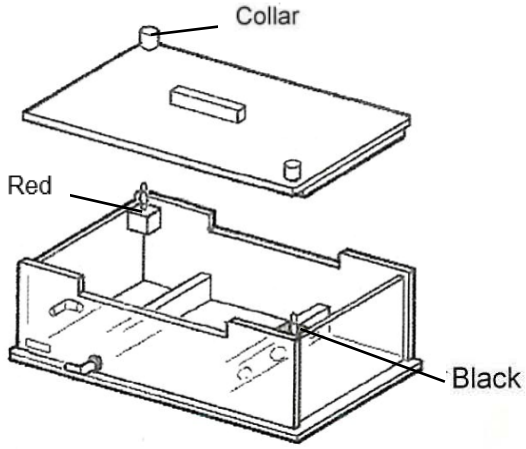
A standard set of #2322178 AE-6111 Agarose EP Kit is composed of the following components.

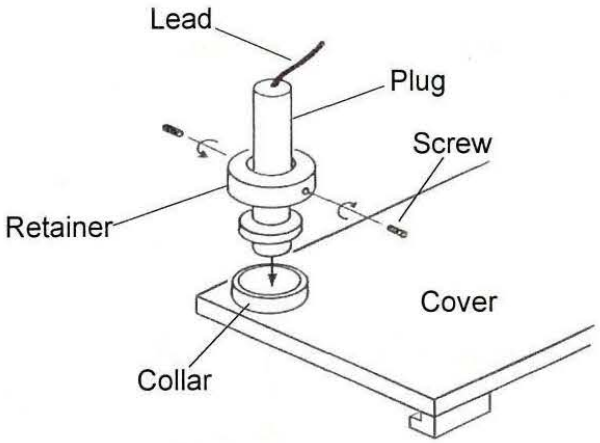
1.	Electrophoresis Chamber	1 pc.
2.	Safety Cover	1 pc.
3.	Gel Tray (for 12 × 16 cm gel)	1 pc.
4.	Drain Stopper (yellow cap attached to electrophoresis chamber)	2 pc.
5.	Power Input Leads (with male & female plugs and a retainer)	1 pair (black & red)
6.	Allen Key	1 pc.
7.	Well Forming Comb (for 13 and 26 wells)	1 pc.
8.	Comb Holder (with 2 plastic screws)	1 pc.
9.	Gel Cast Dam	2 pc.

Section 2. Specifications

Gel size	:	12 cm (width) × 16 cm (length)
Gel thickness	:	Up to 8 mm
Sample well	:	26 wells or 13 wells, per comb
Well forming comb:		
Tooth thickness	:	1 mm
Tooth length	:	12 mm
Tooth width	:	3 mm for 26 wells & 6 mm for 13 wells
Buffer volume	:	700 to 1200 mL
Material	:	Acrylic plastic, except: platinum for electrodes, polypropylene for combs, silicone rubber for gel cast dams & tubing (optional), rubber for drain stoppers, polycarbonate for safety cover,
UV transparency of gel tray	:	≧ 80% to UV ≧ 300 nm
Tubing (optional) diameter	:	7 mm i.d. – 11 mm o.d.
Dimensions (W×D×H)	:	28.6 × 15 × 8.3 cm approx.
Net weight	:	1.5 kg approx.

Section 3. Fix Power Input Leads to Safety Cover





Caution: Prior to use, the power input leads must be fixed to the safety cover, so that electricity cannot be applied unless the electrophoresis chamber are covered by the cover, for safety.

The electrophoresis chamber has 2 electrode terminals, where 1 pair of power input leads will be connected respectively. Note that the electrode terminals for anode is colored red and the electrode terminal for cathode is colored black. **The safety cover** has 1 pair of vertical, cylindrical openings, where 1 pair of power input leads will be fixed respectively. **The power input leads** are colored red and black respectively. Note that the red lead has a female plug marked [+] and the black lead has a female plug marked [−].

Fix Input Leads:

Temporarily place the safety cover on the electrophoresis chamber, while matching the openings of the cover to the electrode terminals of the chamber. Identify one of the openings to correspond to the red, anode terminal and another opening to correspond to the black, cathode terminal. Prepare the power input leads. The retainer of each lead has 2 screws. Once loosen the screws in the retainers of the leads, with the Allen key supplied. Insert the female plugs of the leads into the corresponding openings of the cover respectively, taking care to match the polarities of the plugs and openings. Fit the retainers of the leads over the collars of the openings. Tighten the screws, evenly with the Allen key. Ensure that now the plugs can move in the openings with a range but they cannot be removed from the openings. Note that the plugs are intentionally loose fit so that the female plugs can be smoothly connected to and disconnected from the male electrode terminals of the chamber.

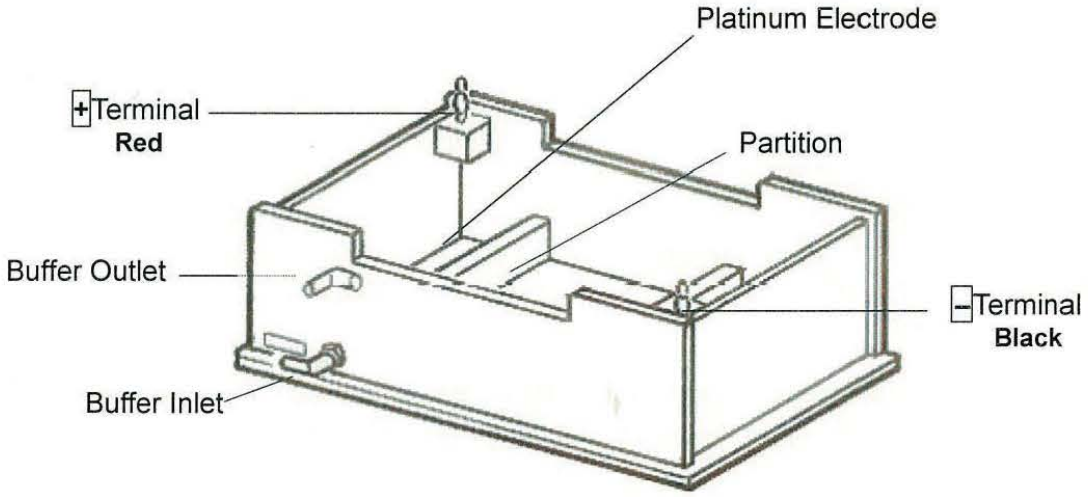
To connect the input leads: Gently push down each female plug of the leads, with fingers, until it stops.

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. Components

4-1 Electrophoresis Chamber:



The electrode terminals (red for anode and black for cathode) connect to the platinum wire electrodes at the inner edges of the chamber.

1 pair of detents on one of the outside walls of the chamber tack the safety cover when the cover once has been placed on the chamber, in order to prevent the cover from being removed accidentally.

1 pair of partitions in the chamber forms 3 compartments, i.e. an anode-side compartment, a compartment under the gel tray and a cathode-side compartment. They prevent buffer under the gel tray from freely moving between the electrodes. The cathode-side partition has holes to allow buffer to move.

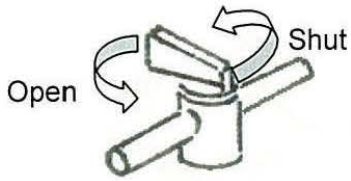
The buffer inlet leads to the compartment under the gel tray, and it allows incoming buffer to flow into the compartment when buffer is recirculated. When the inlet is not used, it is plugged with a stopper (yellow cap). For recirculation of buffer, 7 mm i.d. – 11 mm o.d. tubing (optional) is connected to the inlet port.

The buffer outlet at the end of the anode-side compartment allows buffer to drain out or to flow out when buffer is recirculated. When the outlet is not used, it is plugged with a stopper (yellow cap). For draining or recirculating buffer, 7 mm i.d. – 11 mm o.d. tubing (optional) is connected to the outlet port, and the drain cock is connected to another end of the tubing.

When buffer is recirculated, incoming buffer flows into the room under the gel tray through the buffer inlet, the buffer flows toward the cathode-side compartment through the holes in the cathode-side partition, the buffer flows over the gel tray toward the anode-side compartment, and the buffer flows out from the anode-side compartment through the buffer outlet.

Caution: Power input leads must not be connected directly to the electrode terminals of the chamber. They must be fixed on the safety cover, as per Section 3, so that they are connected through the cover, for safety against electrical hazards.

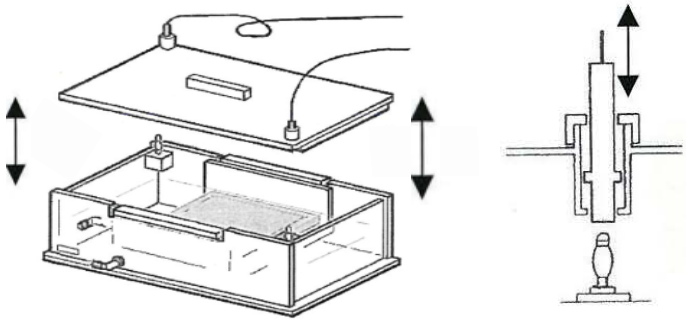
4-2 **Drain Cock** (optional)



To use the drain cock in usual uses, connect 7 mm i.d. – 11 mm o.d. tubing (optional) to drain port (the buffer outlet port) at the bottom of the electrophoresis chamber, and connect the drain cock to the another end of the tubing.

To shut the cock, turn the knob of the cock to a right angle to the flow passage of the cock.
To open the cock, turn the knob of the cock to parallel to the flow passage of the cock.

4-3 **Safety Cover**



Caution: Prior to operation, power input leads must be fixed on the safety cover, as per Section 3, so that they are connected to the electrode terminals of the electrophoresis chamber through the cover, for safety against electrical hazards. Power input leads must not be connected directly to the electrode terminals.

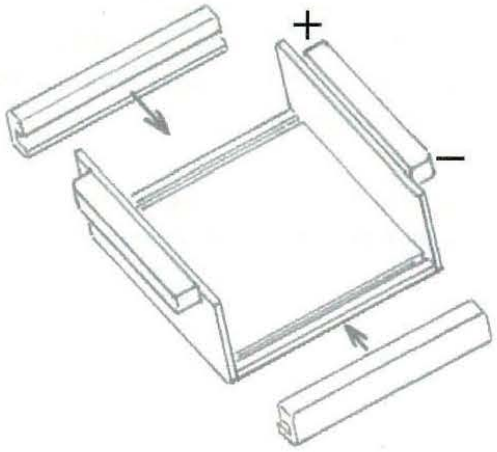
To place the safety cover on the electrophoresis chamber:

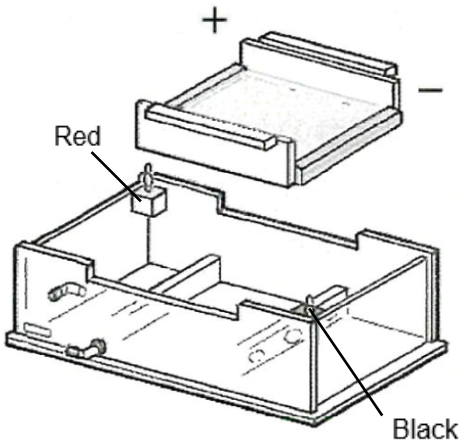
1) While matching the polarities of power input leads with the polarities of the electrode terminals of the electrophoresis chamber, i.e. the red lead to the red anode terminal and the black lead to the black cathode terminal, gently fit the safety cover onto the electrophoresis chamber.
2) Gently push down the female plugs of the power input leads respectively toward the male tips of the electrode terminals until they stop.

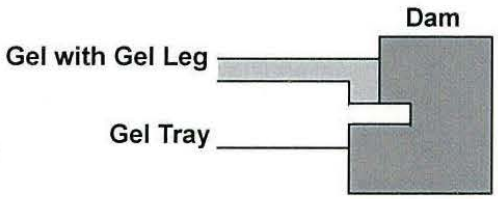
To remove the safety cover from the electrophoresis chamber:

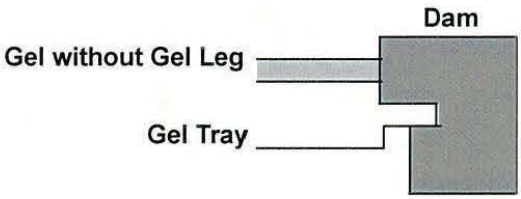
1) While lightly pressing the cover with a hand to prevent the cover from lifting, gently pull up the female plugs of the power input leads respectively to unplug the female plugs from the male tips of the electrode terminals of the chamber.
2) Gently lift off the safety cover.

4-4 **Gel Tray & Dams**









The gel tray has a + mark and a — mark on one of its lengthwise edges. The marks correspond with the red-colored, anode electrode terminal and the black-colored, cathode electrode terminal of the electrophoresis chamber respectively.

The lengthwise edges of the gel tray bear 10 graduations at 1 cm intervals. The graduations indicate distances from the cathodic end of a gel, when a gel has been cast, as a guide for positioning the comb holder on the gel tray, i.e. for positioning a well forming comb.

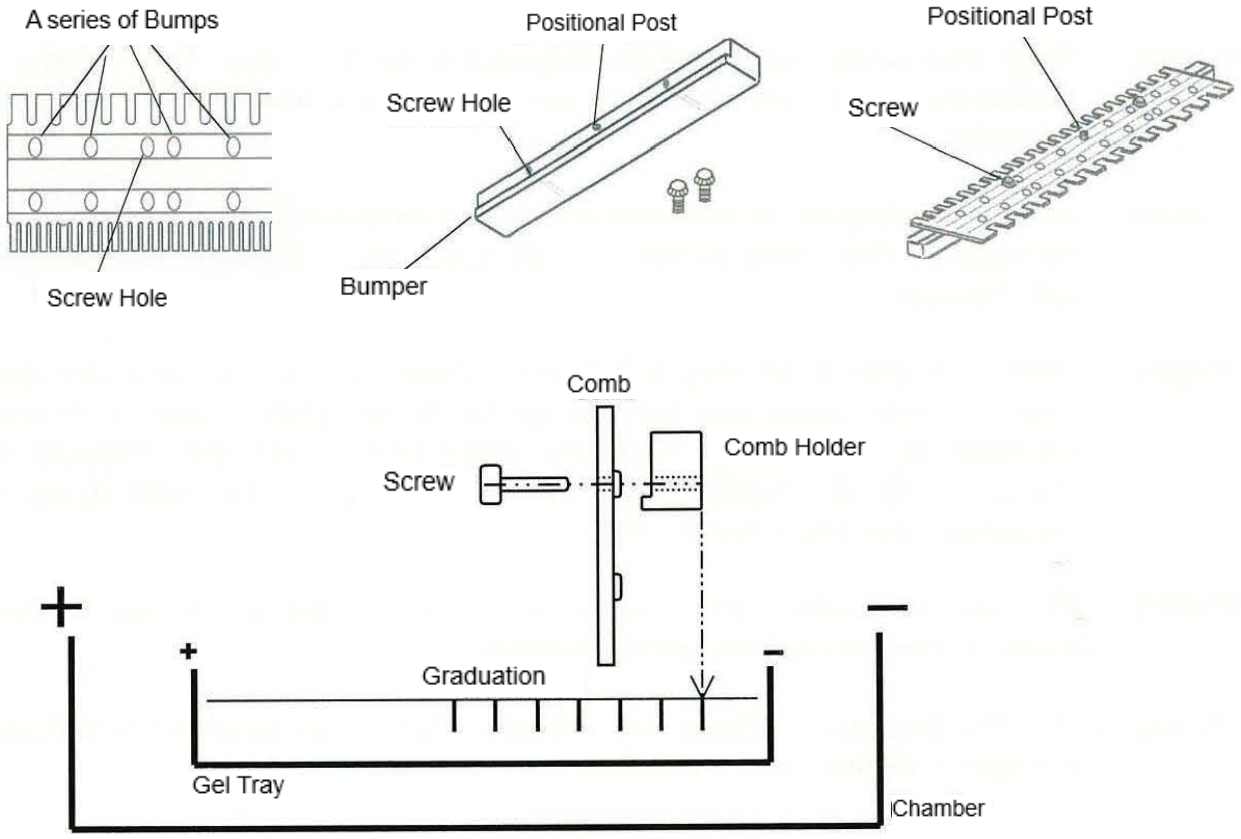
The open, crosswise ends of the gel tray are sealed with the 2 dams of foamed silicone rubber respectively, prior to casting a gel. The ends have thin edges, while the dams have slits. To seal the ends, the thin edges of the gel tray are fit into the slits of the dams.

Note that each of the dams is asymmetric about its slit.

a) With its narrow section upside, a gel leg is formed at the end of a gel. Gel legs prevent a slippery gel from accidentally slipping off the gel tray, or floating in buffer when a gel is set in the electrophoresis chamber.
b) With its wide section upside, a gel leg is not formed, i.e. a smooth, flat gel is cast so that the gel will be easily removed from the gel tray after electrophoresis.

Care should be taken, when fitting the dams to the gel tray, not to allow any minute openings between the ends of the dams and the inner walls of lengthwise sides of the gel tray.

4-5 **Combs** & **Comb Holders**



The comb holder has 2, threaded screw holes and a positional post between the screw holes. It also has a bumper edge at its bottom. 2 plastic screws are attached to the comb holder, when shipped from the factory.

The comb has 13 teeth of a 6 mm width along one of its edges and 26 teeth of a 3 mm width along the opposite edge. The length of the teeth is 12 mm. The intervals of the teeth are compatible with 8-channel multipipets. The comb also has 2 rows of bumps on one of its surfaces. There are 3 holes in each of the rows, one at the middle for the positional post of the comb holder and the other 2 for screws to attach the comb on the comb holder.

To set a comb on a comb holder, the surface of the comb having bumps and the surface of the comb holder having the bumper face with each other. One of the rows of bumps and holes is used to set the comb on the comb holder in order to use the row of teeth which are far from the row for forming sample wells. The bottom of the row of bumps and the top of the bumper of the comb holder are matched, while the positional post of the comb holder is fit in the middle hole in the row. The comb and the comb holder being matched are threaded with the screws.

Note that the comb, having been set on the comb holder, faces toward anode and the comb holder faces toward cathode, in the gel tray, and in the electrophoresis chamber.

Section 5. Operation

Note: Prior to operation, the preceding sections must have been understood. It is desirable to practice use of the components of the apparatus, referring to Section 4, before actually operating the components.

Caution: Avoid environments which may be subjected to air flow, either warm or cool, by air conditioners or such, which may cause uneven gelling of agarose or uneven electrophoretic separation.

Warning: Some of the reagents used in electrophoresis are chemically hazardous. When handling the reagents, care should be taken to protect body and skin against hazardous reagents and chemicals.

Warning: Ethidium bromide is extremely cacerogenic. When handling it, extreme care should be taken to protect human body and skin against the possibilities to touch it, by wearing a laboratory robe and gloves. Care also should be exercised when disposing it. For disposition, refer to: Sambrook, J., *Molecular Cloning*, 2nd Ed., Cold Spring Harbor Laboratory, New York, 6.16-6.17 (1989)

Warning: Electricity may cause severe injury or death. When applying electricity, extreme care should be exercised against electrical hazards.

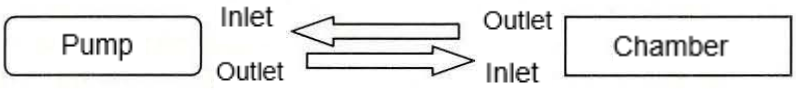
Warning: UV (ultraviolet) rays may injure skin and eyes. Care should be taken not to expose skin and eyes to UV rays, by wearing a face shield or glasses and gloves.

5-1 Preparation of Chamber

5-1-1 **Not to recirculate buffer**:

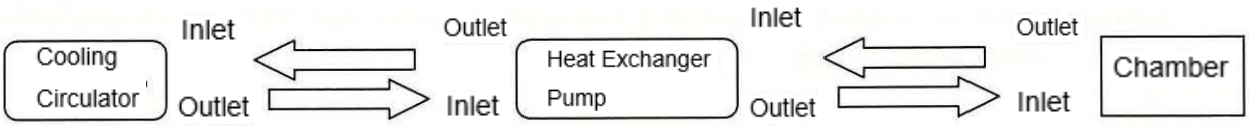
In case the drain cock will not be not used, ensure that both the buffer inlet and outlet is plugged with the stoppers (yellow caps). In case the drain cock will be used for draining buffer after electrophoresis, ensure that the buffer inlet is plugged with the stopper (yellow cap), attach the optional drain tube (i.d. 7 mm – o.d. 11 mm) or an equivalent tube to the buffer outlet, and attach the optional drain cock to the outlet end of the tube. Ensure that the drain cock is shut, i.e. the knob of the cock is turned at a right angle to the flow passage of the cock.

5-1-2 **To recirculate buffer**:



Prepare a liquid circulation pump that can afford a flow rate of about 200 mL per minute. Connect the buffer inlet of the electrophoresis chamber to the liquid outlet of the pump, and the buffer outlet of the chamber to the liquid inlet of the pump, respectively with the optional drain tubes (i.d. 7 mm – o.d. 11 mm) or equivalent tubes. Note that a volume of the recirculation circuit, from the buffer inlet to the buffer outlet through the pump should be counted among a volume of buffer required.

5-1-3 To recirculate and cool buffer



Buffer in the electrophoresis chamber cannot be cooled directly. For cooling the buffer, a heat exchanger needs to be put between a circulation pump to recirculate the buffer and a cooling circulator, in order to cool the buffer through the heat exchanger. A heat exchanger having a built-in circulation pump such as Atto AE-6370 Tempcon is recommended.

Prepare a heat exchanger having a liquid circulation pump that can afford a flow rate of about 200 mL per minute and a cooling circulator. Connect the buffer inlet of the electrophoresis chamber to the liquid outlet of the heat exchanger/pump, and the buffer outlet of the chamber to the liquid inlet of the heat exchanger/pump, respectively with the optional drain tubes (i.d. 7 mm – o.d. 11 mm) or equivalent tubes. Connect the heat exchanger/pump and the cooling circulator with appropriate tubing. Note that a volume of the recirculation circuit, from the buffer inlet to the buffer outlet through the pump should be counted among a volume of buffer required.

5-2 Preparation of Gel Tray

Seal the gel try with 1 pair of dams, by fitting the thin edges of the open, crosswise edges of the gel try into the slits of the dams, either with the narrow section of the dams upside for casting a gel with gel legs, or with the wide section upside for casting a gel without gel legs. Take care not to allow any openings between the ends of the dams and the inner walls of the lengthwise sides of the gel tray, which may leak an agarose solution.

Set the gel tray in the electrophoresis chamber, by laying the lengthwise edges of the gel tray on the lengthwise notches of the chamber, while matching the polarities of the gel tray and the chamber, i.e. the ⊞ mark to the ⊞ mark and the ⊟ mark to the ⊟ mark. It is desirable that the gel tray is level. It is recommended to check level with a level gauge (optional) and adjust level by certain methods such as padding the bottom of the chamber.

5-3 Preparation of Comb

Attach the comb to the comb holder, by matching the bottom of the row of bumps, which is far from the teeth to be used, to the top of the bumper of the comb holder, fitting the positioning post of the comb holder in the middle hole in the row, and screwing 2 plastic screws into the 2 side-holes in the row and the threaded holes in the comb holder.

In case 2 combs are used, attach another comb to another comb holder, in the same way.

5-4 Gelling Agarose

Caution: Avoid an agarose solution of a temperature higher than 60°C, which may deform or damage the gel tray.

Note: When agarose solution has been poured, the comb(s) needs to be set promptly.

Note: In the gel tray, the comb(s) attached on the comb holder(s) should be on the anode side (toward the red electrode terminal) of the electrophoresis chamber, while the comb holder(s) should be on the cathode side (toward the black electrode terminal).

Note: In usual applications, the comb attached on the comb holder would be set 1 or 2 cm away from the cathodic end of an agarose gel. In case 2 combs are applied, the second comb would be set at about the middle between the cathodic and anodic ends of a gel, in order to allow nearly same migration distances for 2 rows of samples.

Note: A gel without gel legs is slippery. When handling the gel tray holding a gel without gel legs, care should be taken not to excessively slant the gel tray, to prevent the gel from slipping off the gel tray.

Gently pour an agarose solution into the gel tray placed in the electrophoresis chamber.

Gently set the comb holder, holding the comb, on the gel tray to immerse the teeth in the agarose solution, while positioning it by referring to the graduations in the lengthwise edges of the gel tray as a guide. In case of using 2 combs, set the second comb holder with the second comb, in the same way.

Allow the agarose solution to gel.

After complete gelation of agarose to opaque white, gently remove the comb(s), by slowly pulling it up slightly aslant.

Take out the gel tray from the electrophoresis chamber. Gently remove the dams, by slowly pulling them off the gel tray.

5-5 Setting Electrophoresis Chamber

Note: When setting the gel tray in the electrophoresis chamber, the polarities of the gel tray and the chamber should be matched with each other, i.e. the ⊞ mark and the ⊟ mark of the gel tray to the ⊞ mark and the ⊟ mark of the chamber, respectively.

Note: When setting the gel tray in the electrophoresis chamber, care should be taken not to trap air bubbles beneath the bottom of the gel tray. Air bubbles beneath the gel tray may cause uneven temperature distribution in a gel, which results in uneven electrophoretic separation.

Ensure that both the buffer inlet and outlet of the electrophoresis chamber have been plugged with the stoppers (yellow caps).

In case the drain tube (optional) with the drain cock (optional) attached to the tube has been attached to the buffer outlet of the electrophoresis chamber, ensure that the cock has been shut.

In case buffer will be recirculated, or recirculated and cooled, ensure that the apparatus for recirculation, or recirculation and cooling, are ready to operate, and that liquid connection of the electrophoresis chamber and the apparatus has been completed.

Gently pour buffer, mixed, into the electrophoresis chamber.

To prevent from air bubbles to be trapped beneath the bottom of the gel tray, once tilt the gel tray to lower either cathodic or anodic end of the gel tray, and slowly immerse the bottom of the lowered end into the buffer until the lowered ends of the lengthwise sides of the gel tray touch the notches of the chamber. Then, slowly lower another end of the gel tray, while releasing air under the gel tray, to immerse the whole bottom of the gel tray in the buffer, until the lengthwise sides of the gel tray sit on the notched sides of the chamber. In the event air bubbles have been trapped, once take out the gel tray and repeat the above operation.

The level of the buffer should be 2 to 3 mm higher than the surface of the gel. If the level is not so, adjust the volume of the buffer.

5-6 Application of Samples

Note: Once samples have been applied, electrophoretic run should be started promptly in order to prevent samples from diffusing.

Gently pipet samples, prepared with marker stain, into the sample wells of the gel.

In case the buffer will be recirculated, start recirculation. The level of the buffer may change when it is recirculated. In the event it changed, adjust the volume of the buffer again so that the level will be 2 to 3mm higher than the surface of the gel.

5-7 Placing Safety Cover

Caution: Withhold connecting to a power supply until the safety cover has been placed.

Slowly lower and fit the safety cover, with the power input leads fixed, onto the electrophoresis chamber, while matching the polarities of the cover and the chamber, i.e. the red plug and the black plug of the cover to the red electrode terminal and the black electrode terminal of the chamber, respectively.

Ensure the female plugs in the safety cover are centered over the male tips of the electrode terminals of the electrophoresis chamber, respectively. Then, gently push down the respective plugs toward the male tips until they stop.

5-8 Connecting Power Supply

Warning: Electricity may cause severe injury or death. When applying electricity, extreme care should be exercised against electrical hazards. Prior to operating a power supply, operation of the power supply must be understood. Especially safety operation against electrical hazards must be thoroughly understood.

Caution: Withhold connecting a power supply to be used to an electric source for the power supply until the connection for electricity between the electrophoresis chamber and the power supply has been completed.

Ensure that a power supply to be used is turned off.

Connect the male plugs of the power input leads of the safety cover to the power outlets of the power supply, while matching the polarities of the leads and the power outlets, i.e. the red plug and the black plug of the leads to the positive ⊞ terminal and the negative ⊟ terminal of the power outlet.

5-9 **Electrophoresis Run**

Warning: Electricity may cause severe injury or death. When applying electricity, extreme care should be exercised against electrical hazards. Prior to operating a power supply, operation of the power supply must be understood. Especially safety operation against electrical hazards must be thoroughly understood.

Warning: When electricity is supplied to the electrophoresis chamber by a power supply, the electrophoresis chamber, the power input leads and any other electrical connectors must not be touched.

Note: During electrophoresis, the electrophoresis chamber should not be exposed to air flow, either warm or cool, by air conditioners or such, which tends to cause uneven temperature distribution in the buffer and lead to uneven electrophoretic separation.

Note: During electrophoresis, care should be taken against a rise of the temperature of the buffer. Temperatures higher than 50°C may deform a gel as well as the gel tray and/or the electrophoresis chamber. In the event of such temperatures, a voltage of input electricity should be lowered, or the buffer should be recirculated, or recirculated and cooled.

Start output of electricity by the power supply.

In usual applications, when the stained markers (the markers of the preceding samples in case of 2 rows of samples) have migrated so that the distances between the markers and the anodic end of the gel are about 1 cm, end the electrophoresis run by stopping the electricity output of the power supply and turning off the main electricity of the power supply.

5-10 **Disconnecting Power Supply** & **Removing Safety Cover**

Ensure that the electricity output and the main electricity of the power supply have been turned off.

Unplug the male plugs of the power input leads of the electrophoresis chamber from the output terminals of the power outlet of the power supply.

While lightly pressing down the safety cover with a hand, gently pull up the respective female plugs in the cover, to unplug the plugs from the electrode terminals of the electrophoresis chamber.

Gently lift and take off the safety cover.

5-11 **Detection**

Warning: Ethidium bromide is extremely cacerogenic. When handling it, extreme care should be taken to protect human body and skin against the possibilities to touch it, by wearing a laboratory robe and gloves. Care also should be exercised when disposing it. For disposition, refer to: Sambrook, J., *Molecular Cloning*, 2nd Ed., Cold Spring Harbor Laboratory, New York, 6.16-6.17 (1989)

Warning: UV (ultraviolet) rays may injure skin and eyes. Care should be taken not to expose skin and eyes to UV rays, by wearing a face shield or glasses and gloves.

Note: A gel without gel legs is slippery. When handling the gel tray holding a gel without gel legs, care should be taken not to excessively slant the gel tray, in order to prevent the gel from slipping off the gel tray.

Gently take out the gel tray from the electrophoresis chamber.

For detection operations of an electrophoresed gel, the gel may be either kept being held in the gel tray or removed from the gel tray. In case of removing a gel, cut off the gel legs, if applied, with a knife or spatula, gently slant the gel tray to slide one of the crosswise ends of the gel onto a flat, and slowly draw off the gel tray, while tilting it, to slide whole the gel onto the flat.

Immerse the gel, with or without the gel tray, in a staining solution. After the gel has been stained, rinse the gel with water. If the background is too thick, decolorise the gel, by immersing it in water.

Apply UV to the gel, with or without the gel tray, to photograph or image it. If the background is too thick, decolorise the gel again, by rinsing it with water.

Section 6. Maintenance

Caution: Organic solvents, high temperature, and/or rigid materials may damage, deform or discolor plastic components. Such should be avoided when cleaning, drying or storing the components of this apparatus.

Caution: The platinum electrode wires in the electrophoresis chamber are very fragile. When cleaning it, care should be taken not to cut or curve them.

After use, clean the electrophoresis chamber, gel tray, dams, combs, comb holders, tubing (if used) and drain cock (if used), thoroughly with running water, and dry them at room temperature. The safety cover also may be cleaned with water. In case of cleaning the cover, once remove the power input leads from it, to avoid wetting the leads and their plugs. After cleaning the cover, do not fail to fix the leads on the cover again.

In the event the components are persistently smeared, clean them with mild detergent and water with the use of a soft sponge, rinse them with running water, and dry them at room temperature. Do not use hard sponges or brushes.

Prior to store the apparatus, ensure that all the components are dry. Especially ensure dryness of its electrical connection parts, such as the power input leads, the plugs of the leads and the electrode terminals of the chamber. It also is desirable to check up any damages or injuries of the electrical connection parts.

Store the apparatus in an environment which are free from direct rays, high temperatures, corrosive gases or dusts.

Replacement Parts:

(Code #)	(D e s c r i p t i o n)
2393 382	Gel Tray, AE-6111 (dams not included)
2393 385	Dams 2/pk, AE-6111
2393 383	1 mm 13 & 26-well Comb, AE-6111
2393 384	Comb Holder, AE-6111 (2 screws included)
2393 330	Drain Stoppers 2/pk
2393 092	Drain Tube (7-11 mm × 1 m)
2393 207	Drain Cock
2392 172	Level Gauge
2328 441	Power Input Leads (1 pair with retainers and 1 Allen key)
2393 381	Safety Cover, AE-6111 (without power input leads)
2393 380	Electrophoresis Chamber, AE-6111
2393 332	Chamber-Temcon Connection Kit (for connecting AE-6370 Tempcon heat exchanger)

Section 7. Technical Guide

Note: Submerged agarose gel electrophoresis is run with a variety of buffer systems, such as TBE (Tris-boric acid-EDTA), TAE (Tris-acetic acid-EDTA), TPE (Tris-phosphoric acid-EDTA) and etc. The following is a guide for electrophoresis with TBE buffer. For other methods, refer to references and papers.

7-1 Reagents

Caution: Some of the reagents used in electrophoresis are chemically hazardous. When handling the reagents, care should be taken to protect body and skin against hazardous reagents and chemicals.

Warning: Ethidium bromide is extremely cacerogenic. When handling it, extreme care should be taken to protect human body and skin against the possibilities to touch it, by wearing a laboratory robe and gloves. Care also should be exercised when disposing it. For disposition, refer to: Sambrook, J., *Molecular Cloning*, 2nd Ed., Cold Spring Harbor Laboratory, New York, 6.16-6.17 (1989)

Note: Reagents of electrophoresis purity or higher purities should be used.

Agarose

Tris (Tris [hydroxymethyl] aminomethane)

Boric acid

Na_2 EDTA

Sucrose

BPB (Bromophenol Blue)

DNA size standards

Ethidium bromide

7-2 Reagent Solutions

7-2-1 **5× TBE buffer stock solution**:

Tris	53.9 g
Boric acid	27.5 g
Na_2 EDTA	3.7 g

Make to 1000 mL with water.
pH adjustment not required.
Store dark at room temperature.

7-2-2 **Running buffer**:

Dilute 5× TBE buffer stock solution to 1/5 concentration (1× TBE) with water.
Prepare and stir when use.

Volume of running buffer:

Gel Thickness	Buffer	Buffer to be recirculated
Up to 3.5 mm	700 mL	800 mL
Up to 6 mm	900 mL	1000 mL
Up to 8 mm	1100 mL	1200 mL

Note: In case buffer is recirculated with a circulation pump or such, a volume of the recirculation circuit, from the buffer outlet of the electrophoresis chamber to the buffer inlet through the pump should be counted among a volume of buffer required.

7-2-3 **BPB marker stain solution**:

BMP 1 mg
Sucrose 600 mg
Make to 1 mL with water.
Store cold and dark.

7-2-4 **Ethidium bromide stock solution**:

Ethidium bromide 100 mg
Make to 10 mL with water.
Store dark at room temperature.

7-2-5 **Ethidium bromide solution**:

Dilute ethidium bromide stock solution to 1/1000 concentration with 1× TBE buffer or water.

7-2-6 **Decoloriser**:

Water.

7-3 **Preparation of Samples**:

Salt concentrations of samples to be electrophoresed in a single gel should be uniform as far as possible. Samples of high salt concentrations disturb migration speeds and patterns for their own lanes as well as neighbor lanes. Salts of samples of high salt concentrations should be precipitated in ethanol and the salt precipitation should be rinsed off. In case size standards are dissolved in TE buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA), dissolve samples in TE buffer. Prior to electrophoresis, mix 1/10 volume of marker stain solutions to 1 volume of sample solutions, to stain and gravitate samples.

7-4 Agarose

7-4-1 Agarose Concentration

Concentrations of agarose would be decided from sizes of DNA fragments to be separated. The table below is a guide. For separation of DNA fragments less than about 500 bp, PAGE is recommended.

Agarose Concentration	Linear DNA Size to Separate
0.4%	5 to 40 kb
0.6%	1 to 20 kb
0.8%	0.7 to 10 kb
1.0%	0.5 to 8 kb
1.2%	0.4 to 6 kb
1.5%	0.2 to 4 kb

7-4-2 Agarose Gel Thickness

Gel thicknesses would be decided from sample volumes to be applied to sample wells. The table below is a guide. For 5 μL samples per well, as an example, a 3 mm gel thickness is necessary, in consideration of a margin and physical strength of a gel.

Sample Volume per Well:

Number of Wells	Tooth Thickness	Tooth Width	Maximum Sample Volume per Well
13 wells	1 mm	6 mm	5.5 μL per 1 mm gel thickness
26 wells	1 mm	3 mm	2.5 μL per 1 mm gel thickness

7-4-3 Agarose Solution

The table below is a guide to decide volumes of agarose solution required.

Gel Thickness	Minimum Volume Required
Up to 3.5 mm	120 mL
Up to 6 mm	150 mL
Up to 8 mm	180 mL

Put buffer of a volume equal to a volume of agarose solution required into a flask. Add weighed agarose: 1.5 g agarose to 150 mL buffer for 1% agarose gel as an example. Mark the level of the buffer and agarose content, in order to compensate evaporation after heating.

Boil the buffer in water bath or a microwave oven until agarose is completely dissolved. Compensate the evaporation loss, by adding water to recover the level marked before boiling. Allow to cool down to 50 to 60°C. Care should be taken for the temperature of an agarose solution, because overcooling makes the solution start gelling while an agarose solution of a temperature higher than 60°C may deform or damage the gel tray.

7-5 **Electricity**

Warning: Electricity may cause severe injury or death. When applying electricity, extreme care should be exercised against electrical hazards.

Note: Electrical conditions are variable according to conditions of electrophoresis. For particular conditions, electricity should be decided from references or experiments.

In usual applications, apply 100 V (4 V/cm approx.) constant voltage × 150 minutes approx. for 1 row of samples or 60 minutes approx. for 2 rows of samples. As voltages to be applied are proportional to distances of electrodes, it is not necessary to adjust voltage dependent on thicknesses or lengths of gels.

Higher voltages provide faster separation. As an example, 150 V (5.8 V/cm approx.) for a 6 mm thick gel affords separation in about 100 minutes. However, high voltages increase Joule heat, and it may cause uneven temperature distribution in a gel, which disturbs separation. Care should be taken not to allow temperature of buffer rise to 50°C or more, irrespective of buffer systems. To prevent electrophoresis from disturbance by excessive Joule heat, voltage should be lowered or buffer should be recirculated and cooled.

At room temperature, current initially is about 65 mA and rises to about 80 mA. In case of 2 rows of samples with the use of 2 well forming combs, apply the same electricity for about ½ time for 1 row of samples. Current and a time of period to apply electricity are varied according to buffer systems, characteristics of agarose, agarose concentrations, gel thicknesses, migration distances aimed and etc.

When the migration front is about at 1 cm to the end of a gel, stop electricity.

7-6 **Staining**

Warning: Ethidium bromide is extremely cacerogenic. When handling it, extreme care should be taken to protect human body and skin against the possibilities to touch it, by wearing a laboratory robe and gloves. Care also should be exercised when disposing it. For disposition, refer to: Sambrook, J., *Molecular Cloning*, 2nd Ed., Cold Spring Harbor Laboratory, New York, 6.16-6.17 (1989)

To stain a gel with ethidium bromide, immerse a gel, either with or without the gel tray, in ethidium bromide solution for 20 to 30 minutes. Care should be taken to ensure the gel is completely immersed in the solution. Take out the gel and rinse it with water. In the event the background is thick, immerse the gel in water as decolorisation solution for 5 to 30 minutes.

7-7 **Detection**

Warning: UV (ultraviolet) rays may injure skin and eyes. Care should be taken not to expose skin and eyes to UV rays, by wearing a face shield or glasses and gloves.

For detection of DNA, UV of wavelengths ≥ 300 nm are recommended. To apply UV ≥ 300 nm, to which the gel tray is about 80% transparent, place a gel, either with or without the gel tray, on a UV transilluminator, and observe, photograph or image the gel.

In case of applying 254 nm UV, which damages DNA, care should be taken not to expose a gel to the UV for a long time. To apply 254 nm UV, remove a gel from the gel tray and place the gel on a UV transilluminator, or irradiate the UV down to the gel kept being laid on the gel tray, and observe, photograph or image the reflection of the gel.

Warranty

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

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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.
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7. The warranty period is one (1) calendar year from a date when the products are shipped from Atto to an original purchaser.
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ATTO CORPORATION
3-2-2 Motoasakusa,
Taito-ku, Tokyo
111-0041 Japan
Tel; +81-3-5827-4863
Fax: +81-3-5827-6647
e-mail: eig@atto.co.jp
https://www.attoeng.site/

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