# Deluxe Electrophoresis Chamber

36 W 5190

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WARD'S Natural Science

# Agarose Electrophoresis System Operations Manual

Nucleic acids, both DNA and RNA, can be separated on the basis of size and charge by means of electrophoresis, a very important technique in molecular biology. Electrophoresis is used to determine the quantity and quality of DNA and RNA, as well as the sizes of DNA and RNA molecules, providing important information about the size of genes and mRNA molecules.

Electrophoresis is the process by which molecules are forced across a gel by an electrical current. The electrical current from one electrode repels the molecules, while the other electrode attracts, and the frictional force of the gel material acts as a "molecular sieve", separating the molecules by size and charge. Negatively charged molecules will migrate toward the positive pole, while positively charged molecules will migrate toward the negative pole. The net negative charge of the phosphate groups, which are located in the "backbone" of the nucleic acid structure, results in the DNA fragments having a negative charge and thus will always migrate toward the positive pole. After staining, the separated macromolecules in each lane can be seen; they appear as a series of bands spread from one end of the gel to another.

The most commonly used medium for nucleic acid separation is agarose, a natural colloid extracted from seaweed. Agarose is used often because agarose gels have a very large pore size. The size of these pores depends on the concentration of agarose used; typically the concentration varies from 0.5% to 2.0%. The lower the concentration, the larger the pore size of the gel and the larger the nucleic acid fragments that can be separated. The size of the pores in an agarose gel are relatively homogenous. As a result, the larger nucleic acid fragments move more slowly through the gel than do smaller DNA fragments.

## Equipment and Materials

WARD'S Electrophoresis Chamber is a horizontal electrophoresis tank (Figure 1), 10.7 x 4.2 x 3.2 cm, that holds two acrylic gel casting trays and uses platinum wires for superior electrical conductance.

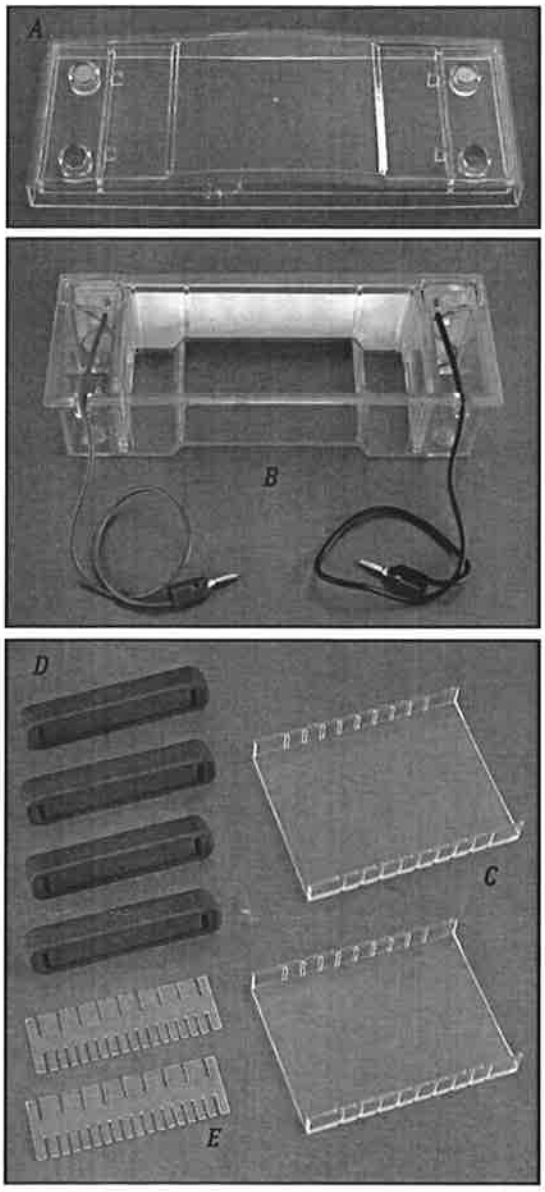


*Figure 1*

## Included:

A) 1 Magnetic Safety Cover
B) 1 Electrophoresis Chamber with Attached Electrode Leads
C) 2 Gel Casting Trays (also available separately, 36 W 5188)
D) 4 Rubber Tray End Dams (also available separately, 26 W 5189)
E) 4 8- and 16-Well Gel Combs (also available separately, 36 W 5187)

## Agarose Gels

Agarose is a natural polysaccharide of galactose and 3,6-anhydrogalactose extracted from seaweed. The ability of an agarose gel to act as a sieve and filter, or resolve, a particular nucleic acid fragment (or whole plasmid) depends on the agarose concentration.

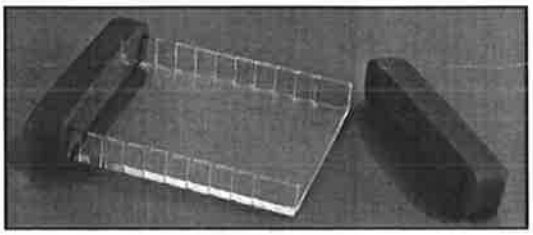

*Figure 2*

1. Fit the end dams onto each end of the gel casting tray, as shown in *Figure 2*. The end dams seal the ends of the tray, eliminating the need to tape the ends of the tray.

2. Insert the gel comb, using either the 8-well or the 16-well side, into the slots near the end of the tray (DNA) or the center slots of the tray to separate negatively and positively charged molecules.

   Since the DNA is negatively charged, if separating DNA, the tray is placed in the tank with the wells closest to the negative electrodes, so the DNA fragments will migrate across the gel toward the positive electrode. *See Figure 3.*

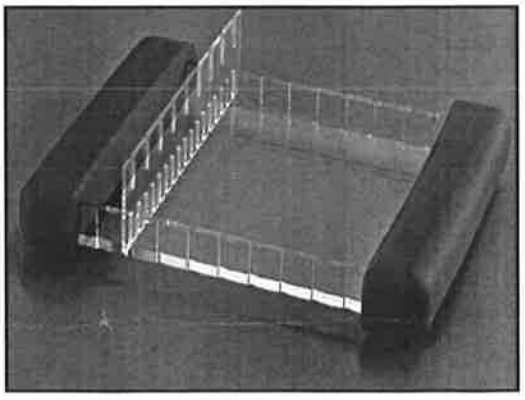

*Figure 3*

> ***Note:*** *For easier removal of the gel comb from the solidified agarose gel, you may want to coat the comb with a small amount of petroleum jelly before inserting it onto the gel casting tray.*

## 4

## *Casting an Agarose Gel*

Agarose can be prepared either by using a melted, pre-cooked (prepared) formulation of a known agarose percentage, or by preparing a formulation. Refer to Appendix II in WARD'S Electrophoresis System Manual (32 W 0807) for formulations. All WARD'S gel electrophoresis kits contain pre-cooked agarose, in various percentages. Each 200 ml bottle will produce approximately eight 25 ml gels. Refer to Appendix I in the manual for a list of the percentage agarose used each kit.

1. Melt pre-cooked agarose with a hot water bath or a microwave.

   ***Safety: Always handle agarose bottle with heat-protective gloves.***

   - **Water Bath:** Loosen cap on agarose container before placing in water bath. Water should be at least 100°C.
   - **Microwave:** Loosen cap on agarose container before microwaving. Heat in one-minute intervals on low power until agarose is melted.

     ***Note:*** *If, when heating in a microwave, agarose starts to boil before melting completely, stop microwave, swirl agarose bottle, and continue.*

2. After agarose is melted, pipet or measure, using a clean graduated cylinder, 25 ml of agarose.
3. Pour agarose into the gel casting tray in the casting tray holder.

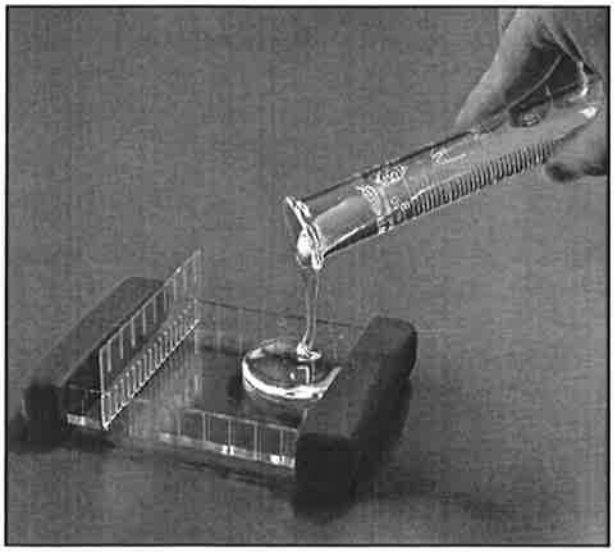

*Figure 4*

4. Allow the agarose to solidify for approximately 20 to 30 minutes. Do not disturb the gel tray or comb. When the agarose has solidified, it will turn opaque.
5. Remove the comb and gel casting tray from the holder by opening the sides of the holder while lifting up the casting tray. Leave the gel on the tray.

> ***Note:*** *Gels may be used immediately, or may be stored in a refrigerator for later use. When storing gels, cover with 1x buffer and plastic wrap to prevent desiccation. Do not store gels for longer than two weeks.*

## *Sample Preparation*

### Fragment Imaging

DNA fragment imaging depends on the type of gel and the type of nucleic acid stain used. Generally, agarose gels are used to image DNA fragments ranging in size from 200 to 50,000 bp. See Appendix III in WARD'S Electrophoresis System Manual for more information. PAGE (polyacrylamide) gels, though predominantly used for lower-molecular-weight proteins, may be employed to separate DNA fragments. Because this type of gel contains extremely fine molecular gradients, it can only separate DNA fragments smaller than 6,000 bp.

### DNA Samples

Read the label and technical information enclosed with each sample before proceeding. Each label provides information regarding the total amount of DNA (expressed in μg) supplied. Refer to Appendix III in WARD'S Electrophoresis System Manual for additional information on WARD'S DNA samples.

Samples may be refrigerated for up to one month. To store samples longer, keep frozen at -3°C, and thaw in a refrigerator for approximately one hour.

### Loading Dye

Loading dye contains sucrose, which causes to DNA sample to sink to the bottom of the well; bromophenol blue, the tracking dye that creates a visible trail as the sample moves down the gel; and TE buffer, matched to that of the gel to assure correct pH. Bromophenol blue is equivalent to a 250 bp fragment when run on a 1.2% agarose gel. See Appendix II in WARD'S Electrophoresis System Manual for more information on loading dye, and Appendix II for more information on loading dye base-pair equivalents.

> ***Note:*** *It is not necessary to add loading dye to samples in WARD'S gel electrophoresis kits, as all samples have the dye added.*

### Reaction Tubes

To transfer DNA from larger samples to individual microcentrifuge "reaction" tubes (usually 10µl; refer to individual experiments for specific requirements), use either a variable volume micropipet, fixed volume micropipet, or a student capillary micropipet. Appendix III of WARD'S Electrophoresis System Manual contains additional information on using micropipets.

## *Heating Samples*

If a procedure requires a DNA sample to be heated to improve band separation, you may heat the sample one of two ways:

### Option 1

All WARD'S gel electrophoresis kits' DNA samples are supplied in a gray sample box which can be used as an inexpensive incubator. Place the microcentrifuge reaction tubes containing samples and loading dye in the box. Fill the box with distilled water heated to approximately 90°C until the water level reaches the clear plastic dividers. Do not overfill the box. Cover the box and allow the samples to incubate for five minutes.

### Option 2

Using either a Round Bubble Rack (18 W 4215) or Microcentrifuge Rack (18 W 4205), immerse the tubes halfway in a 90°C water bath for five minutes.

## *Gel Loading*

1. Remove specified volume of sample from the cast gel in the gel tray with a clean micropipet.
2. Transfer the sample to a gel well, taking care not to pierce the bottom of the well with the micropipet tip. Do not overload wells.
3. Repeat the above procedure until all samples have been loaded.

## *Running a Gel*

1. Make sure that the power supply to be used with the electrophoresis chamber, including power cord and patch cords, are away from any moisture. For safety, the apparatus *(Figure 5)* should be in a designated area of your laboratory, away from everyday activities.
2. Place each loaded gel, on a gel tray, in the center of the electrophoresis chamber. Position the well-side of the gel near the negative electrode.

3. Add 1X TBE running buffer, approximately 575 ml, to one of the side wells of the chamber until the level of buffer is approximately 2mm above the top surface of the gel. Do not overfill the chamber with buffer.

   ***Note:*** *If using a WARD'S gel electrophoresis kit, the 500 ml TBE running buffer concentrate (5X) included with the kit must be diluted. Add 57.5 ml concentrate to 517.5 ml distilled water to obtain 575 ml of 1X buffer.*

4. Making sure the cover is dry, lower it onto the electrophoresis chamber. Wipe off any spills on the apparatus before proceeding to the next step.

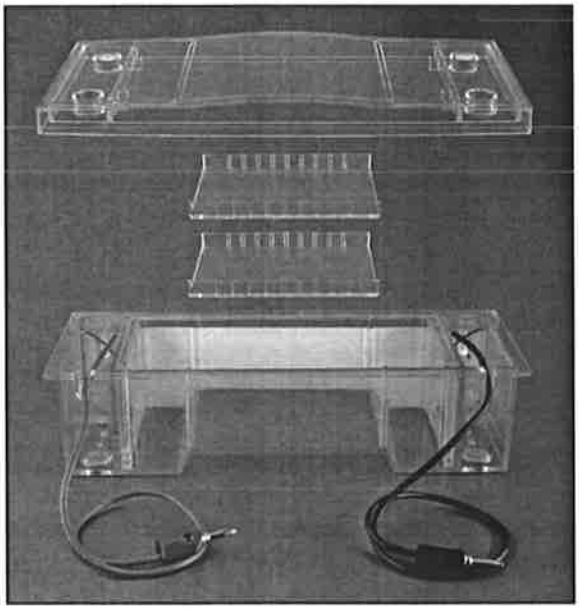

*Figure 5*

***Important! Make sure power supply is unplugged!***

***Note:*** *If using WARD'S Deluxe Power Supply, refer to the special operating instructions that follow.*

5. Insert the positive (red) patch cord to a positive port of an appropriate power supply. Insert the negative (black) patch cord to the sister negative port of an appropriate power supply.
6. Plug the power cord of the power supply into standard 120V outlet.
7. Turn on the power supply. The red power light will illuminate, and bubbles will form along the platinum electrodes. Set the voltage adjustment knob to desired voltage, depending on the number of electrophoresis chambers that are connected to the power supply and the migration rate of the samples.
8. Observe the migration of the sample down the gel toward the red (positive) electrode. Turn off the power and unplug the unit when the loading dye has reached the end of the gel.
9. To ensure the utmost safety, wait approximately 10 seconds before disconnecting the patch cords from the power supply.
10. Remove safety cover and gel tray(s).
11. Carefully push the gel off the gel tray and into the staining tray with a gel handler.

## *Gel Staining*

There are many types of DNA stains available to image separated DNA fragments. Silver nitrate, a very sensitive stain, can image minute amounts of DNA at the nanogram level. It should be noted, however, that the staining process is similar to that of wet-plate photography; it is complicated, and requires the use of hazardous chemicals.

Ethidium bromide is an excellent stain for DNA; however, it is a carcinogen, requiring the use of extensive personal protective equipment, as well as a UV light source that fluoresces the stain. A photographic image must be taken to analyze the DNA.

Methylene blue, a biological stain, is most often used when imaging DNA in an educational setting, since it requires no hazardous chemicals. However, because the imaging of DNA fragments is rather poor, with methylene blue staining both the gel matrix and DNA, a light table is usually required to view small fragments.

WARD'S DNA stain, which can be used with either agarose or PAGE gels, provides an alternative to these stains, requiring no other chemicals and producing clear DNA fragment band imaging.

The amount of DNA placed in a well of an agarose gel must match to the staining requirements. The DNA sample is distributed proportionally within separated fragments; larger top fragments have a greater base-pair length, (more DNA) than smaller bottom fragments. Generally, 1.0 to 1.4 µg per well must be used for methylene blue or WARD'S DNA stain. Ethidium bromide will image 0.2 to 0.5 µg DNA, silver nitrate less than 0.1 µg. Unless otherwise noted, all samples used in WARD'S gel electrophoresis kits are provided in 10 µg amounts—enough for eight sample lanes at 1.25 µg per lane. If a 10 µg sample is heavily diluted, i.e., to 0.5 µg per sample, and methylene blue or WARD'S stain is used, there will be minimal fragment imaging.

Instructions for staining separated DNA fragments are supplied with each type of stain and each lab activity; follow the specific directions for each procedure.