

# Getting Started With Gel Electrophoresis



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## INTRODUCTION

Welcome to our presentation on introductory gel electrophoresis. With the extensive range of equipment now available for schools, gel electrophoresis can be performed safely by science students in school laboratories.

## WHAT IS GEL ELECTROPHORESIS?

An electric field provides the motive force to drive charged molecules through a gel. The porosity of the gel determines how quickly the molecules move. Smaller molecules go further than larger ones in a given time. Gel electrophoresis has become the standard technique for separating mixtures of charged molecules such as proteins and fragments of DNA.

By selecting the right combination of electric field strength, gel type, and gel concentration, complex mixtures can be separated and the individual components can even be collected for further analysis.

Let's get started by looking at how to set up and use the equipment to perform electrophoresis on DNA.

## THE POWER SUPPLY

Power supplies generally run up to four gels simultaneously. An adjustable control allows you to select a suitable voltage (DC). Generally, a built-in safety switch limits current to no more than 100mA per channel.

## THE GEL ELECTROPHORESIS CHAMBER

Electrophoresis chambers consist of:

- a tank fitted with wire electrodes, usually platinum
- a lid with interlocking electrical connections and leads
- a gel casting tray with provision for central or end location of the comb
- a comb to give, typically, 8 wells per gel, each 10 $\mu$ L in volume.

## PREPARING TBE BUFFER SOLUTION

Tris Borate EDTA (TBE) buffer is usually supplied as a 10x or 20x concentrate. Prepare diluted (1x) working solutions as required. Re-dissolve any solids that precipitate out during storage by heating in a water bath.

**NOTE:** *The purpose of the buffer is to provide a low conductivity solution to channel the electric field. TBE is designed to keep DNA stable under the conditions of electrophoresis.*

## PREPARING A GEL

To make a 0.8% agarose gel, sprinkle 0.8g of agarose powder into 100mL of TBE buffer in a conical flask. Heat to the boil in a microwave oven, then allow to cool to 60°C.

**NOTE:** *A hot plate may be used for making larger quantities of agarose solutions, but keep the mixture stirred at all times.*

*For short term storage (up to several hours), gel solution can be kept in a liquid state in a hot water bath at 55-60°C. Keep the container covered to minimize loss of moisture.*

*For long term storage, excess gel can be sealed and kept in a heat resistant container for later use. Simply re-melt the gel for pouring.*

*For your personal safety and to avoid cracking the gel casting tray, never pour the agarose before it has cooled to 55-60°C.*

Seal each end of the casting tray with masking tape and place the comb into position. Put the tray on a flat and level surface.

**NOTE:** *Use the end position for the comb when carrying out electrophoresis of DNA. Use the middle position when separating samples that contain both positively and negatively charged molecules, for example food dyes, microscopy stains, marker pen inks.*

Carefully pour the cooled agarose solution into the tray. Fill to approximately two thirds of the height of the teeth of the comb.

**NOTE:** *Avoid forming bubbles in the gel by gently pouring the agarose solution into the end of the tray away from the comb*

Leave undisturbed until the gel has set, then carefully remove the comb and masking tape.

**NOTE** *Take care to keep the tray horizontal or you risk having the gel slide off once the comb and tape have been removed.*

*You can prepare gels up to a few days in advance. Simply wrap them in cling wrap and store in the refrigerator until required.*

The gel can now be placed in the tank with the wells oriented to the negative electrode. Pour TBE buffer solution into the tank until it just covers the surface of the gel. Ensure there are no bubbles trapped in the wells.

**NOTE** *Use no more TBE buffer than required to cover the gel. Excess buffer reduces the electric field strength and can cause slower DNA migration and band distortion.*

*Ensure the buffer has been diluted to 1x. Using a concentrated buffer solution will reduce the electric field strength (by increasing conductivity).*

*Take care to orient the gel correctly. If a gel is run backwards, even for a short period, the samples are usually lost.*

## USING A MICROPIPETTE

If you are doing preparation and mixing work, you'll probably need a variable volume micropipette. However, these can be expensive, so for routine student work, we recommend fixed volume micropipettes. These are suitable when the DNA samples have been pre-mixed with buffer and are ready to load.

- Fit a clean tip to the barrel of the micropipette.
- To draw up a sample, depress the plunger fully and immerse the tip in the liquid to be collected. Use a slow and controlled action to release the plunger and draw the liquid into the tip.
- To expel a sample, place the outlet of the pipette tip below the surface of the buffer and over the desired well in the gel. Use a slow and controlled action to depress the plunger fully and release the contents into the well.
- Carefully withdraw the micropipette, holding the plunger in the down position until clear of the liquid.

Repeat these steps for each well until all samples have been loaded.

**NOTE** *If you have less than eight samples to run on a gel, fill the central wells in preference to the wells at the edges.*

*Use smaller rather than larger volumes for sharper bands. The standard comb supplied with the BioEd electrophoresis chamber is designed for a volume of 10 $\mu$ L.*

*The wells can be difficult to see on a light background. Place a piece of black card under the chamber to highlight the position of the wells*

## RUNNING THE ELECTROPHORESIS

- When the wells have been loaded and you are ready to begin, carefully fit the lid of the chamber by sliding it into the grooves in the tank. Ensure the electrical plugs line up with the sockets on the tank.

**NOTE** *Never force the lid into position. If you encounter resistance, check that the plugs and sockets are aligned and try again.*

- Plug the leads into the power supply, then choose a suitable voltage setting and turn on the power.

**NOTE** *It is usual to start at a setting of 100V. This causes the samples to move quickly into the gel where they cannot be disturbed. The voltage can then be reduced to suit the time available. The following can be used as a guide:*

- *100V will generally give a run time of 30 – 60 minutes*
- *10V will be suitable for running a gel overnight*
- *Set the voltage at 50V for a run time of around 3 hours.*

*The power setting chosen will apply to all chambers connected to a particular power supply, but you can connect or disconnect a chamber without affecting the others. For safety, it is recommended that you briefly turn the power supply off whilst changing a connection.*

*To ensure the unit is receiving power, check for the evolution of gas bubbles at the electrodes due to electrolysis.*

*The liquid in the chamber will warm up during the process, and some condensation is likely to form on the underside of the lid.*

- Monitor the progress by following the position of the loading dye. Switch off the power when the loading dye is about three quarters of the way down the gel.

**NOTE:** *The loading dye, bromophenol blue, will generally run at the same rate as a DNA fragment of around 200-400 base pairs.*

- Remove the lid of the chamber and lift out the gel and tray. Carefully slide the gel into a shallow dish.

**NOTE:** *Check that the temperature of the buffer is comfortable to touch before attempting to remove the gel.*

*The gel will be quite slippery at this point so take care to keep the tray horizontal until you are ready to deal with it.*

*The buffer can be retained for reuse up to three times.*

## STAINING THE GEL

- Use a plastic pipette to cover the upper surface of the gel with a small volume of concentrated stain solution.
- Allow the stain to soak into the gel for 5 minutes then rinse off excess stain with tap water.
- Seal the dish and gel in cling wrap for several hours to allow the stain to develop.

**NOTE:** *It is often convenient to allow this step to proceed overnight.*

- To heighten the visual contrast between the DNA bands and the background stain, you can perform a de-staining step. To do this, cover the gel with water and occasionally agitate to leach out unbound stain.

## STORING THE GEL

Place the gel in a press-seal bag with a few drops of dilute stain solution. Store in the refrigerator and handle with care. For long term storage, use 70% ethanol.

**NOTE:** *A light box can help show up the stained bands. You can make a record by capturing an image with a camera or a scanner.*

## ELECTROPHORESIS WITH DYES

Water soluble dyes offer a good opportunity to demonstrate the way electrophoresis works and to gain experience with the equipment and techniques.

Dyes require very little preparation. Before loading, just mix each dye with an equal volume of glycerol or 40% sucrose solution. This increases the density of the solution to make it easier to load the dyes into the wells.

**NOTE:** *An alternative method is to soak a small piece of filter paper in dye solution then place it in a well. This method is particularly useful if you are going to work with marker pen dyes. With this method, you pour the buffer solution into the chamber after the wells have been loaded.*

For dyes, a simple solution of NaHCO<sub>3</sub> in distilled water can be used as an effective buffer. Prepare a 1% stock solution and let this down to a 0.1% working solution as required.

Rather than agarose, inexpensive plain agar can be used as the gel medium. Dissolve 1g of agar in 100mL of 0.1% NaHCO<sub>3</sub> buffer. Place the comb in the middle position to separate mixtures of positively and negatively charged dyes. Unused gel solution can be stored at room temperature and "re-melted" on a later occasion.

Dyes are visible in the gel so you can see the separation without having to perform an extra staining step.

### **USING THE GEL LOADING PRACTICE KIT**

The gel loading practice kit lets you load wells as often as you like without wasting reagents and materials. The synthetic resin gels can simply be rinsed under the tap and reused when you want to start again. There is even a realistic DNA electrophoresis transparency that you can use to explain the process.

**NOTE:** See product code G40.64 in our on-line catalogue.

### **FURTHER INFORMATION**

The key to good results with gel electrophoresis is familiarity with the equipment and confidence in the techniques. For more information, contact us or attend a hands-on practical workshop session at a forthcoming conference or seminar. See the "Events" section of our web site for times and locations.