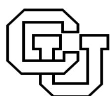

Biological Sciences Initiative

Gel Electrophoresis – Teacher Instructions

Suggested Grade Level: Grades 7-14 **Class Time Required:** 1 period (50 minutes)

EQUIPMENT AND MATERIALS NEEDED (per group)

Electrophoresis chamber, gel form and comb
Electrophoresis power supply
Agar gel
Salt solution
Scientific stains to use as samples
Sample-loading device
Masking tape, if needed to seal gel form

GEL ELECTROPHORESIS BRIEF PROCEDURE

See the MATERIAL PREPARATION GUIDE for preparation of specific materials and for a detailed description of each of these steps.

1. ***Dissolve the agar, cool the solution, and pour the gel.***
Combine agar and water. Bring the mixture to a boil and heat until the agar is dissolved.
Cool the agar until you can comfortably touch the flask.
Place tape across the ends of the gel form (if needed) and place the comb in the form.
Pour cooled agar into the form; the bottom 1/3-1/2 of the comb should be immersed.
Immediately rinse and fill the agar flask with hot water to dissolve any remaining agar.
When the agar has solidified, carefully remove the comb.
Remove the tape (if used) from the ends of the gel form.

2. ***Load samples in the wells in the gel.***
Make a written record of which sample you will load in each well of the gel.
Place the gel form on a black or dark surface to help you see the wells in the agar.
You may find it helpful to only load samples in every other well.
Be careful to not puncture the bottoms of the wells

3. ***Place the gel in the electrophoresis chamber with the wells closest to the negative (black) electrode.***

4. ***Prepare the salt solution and add it to the chamber.***
Add salt to tap water and swirl it to dissolve.
Fill each half of the chamber, adding solution until it is close to the top of the gel. Then gently flood the gel from the end opposite the wells to minimize sample diffusion.

5. ***Place the lid on the chamber and connect the electrode leads to the power supply.***
Connect the black lead to the negative terminal and the red lead to the positive terminal.

6. ***Turn on the power supply and adjust the voltage to 50-100 volts.***

7. ***Run the gel for 5-10 minutes.***
You will be able to observe the samples separating into different colors.
Placing the chamber on white paper will help you see the color separation.

8. ***Turn off the power supply, disconnect the electrode leads, and remove the chamber lid.***

9. ***Remove the gel from the electrophoresis chamber.***
You may also remove the gel from the form and place it on a piece of plastic wrap.
Placing the gel on a piece of white paper will help you better see the results.
Record and evaluate the results of the electrophoresis.

10. ***Clean up.***
Discard the gel in the trash and pour the salt solution down the drain.
Rinse the electrophoresis chamber and gel form with tap water; turn them upside down to dry.

MATERIAL PREPARATION GUIDE

AGAR GEL

Background - Agar is a gelatinous substance found in the cell walls of certain red marine algae, particularly those in the genus *Gelidium*. It is used to make vitamin and drug capsules, as a dental impression material, as a base for cosmetics, and as a culture medium for bacteria and other microorganisms. In foods, it is used as a moisture retention (antidrying) agent in bakery products, in the preparation of rapid settling jellies and desserts, and as a temporary preservative for meat and fish in tropical regions. While a very expensive, highly purified form of sugar, called agarose, is needed for separating DNA or proteins in gels, several kinds of agar can be used for separating scientific stains in gels. These less expensive agars include agars used for microbiological culture media and agars available from health food stores and Asian markets. (Note: Unflavored gelatin, such as Knox®, does not work. The gelatin has a greater affinity to surfaces than to itself, causing the wells to tear out of the gel when the comb is removed. Also, gelatin melts at a much lower temperature and a gel prepared from gelatin is likely to melt while running.)

Agars that can be used for electrophoresis:

Product	Manufact-urer	Source	Cost	Notes
Agarose	Various	Biological supply co.	$8.95/5 grams	Can be used to separate DNA or scientific stains
Bacto-Agar	Difco	Bio supply co.	$71.20/100 grams from VWR	Usually used to make microbiological media. Can be used to separate scientific stains.
Agar	Unknown	Health food store	$.4.28/oz (28.4 g/oz)	Can be used to separate scientific stains.

Cost: Based on listed catalog prices June, 2003.

Agar Preparation Methods

Materials needed:

Beaker or Erlenmeyer flask
Graduated cylinder
Plastic wrap or aluminum foil
Agar
Tap water
Balance
Pot holder or paper towel
Gel form and comb
Stir bar (optional)
Microwave oven or heat source such as stirring hotplate, Bunsen burner, etc.
Masking tape, if needed to seal gel form

Procedure:

1. Choose a beaker or Erlenmeyer flask that is 2-4 times the volume of the solution
2. Add the tap water (10 mls per gel)
3. Weigh the agar and add it to the water (0.1 g agar per gel).
4. Dissolve the agar using one of the following methods:

Microwave method:

Cover the container with plastic wrap; pierce a small hole in the plastic for ventilation.
Heat the solution in the microwave oven on high power until it comes to a boil. Watch the solution closely; cheap agar foams up and boils over easily.
Remove the container (protect your hand with a pot holder or paper towel) and gently swirl it to re-suspend any settled agar.
Reheat the solution until it comes to a boil again.
Remove the container and gently swirl it, looking to see if the agar is dissolved.
If the agar is not dissolved, continue to heat and swirl the solution until dissolved.

Heat Method:

If using a stirring hotplate, place a stir bar in the beaker or flask.
Cover the beaker or flask with a piece of aluminum foil.
Weigh the beaker or flask containing the solution (optional).
Bring the solution to a boil while stirring (or occasionally swirl the container).
Lower the heat and simmer (continue stirring or swirling) until the agar dissolves.
Re-weigh the container, or pour the hot liquid agar solution into a graduated cylinder.

Add hot tap water to replace any water lost during the heating process; mix thoroughly.

5. Cool the solution to 55 C (until you can comfortably place your hand on the container). The covered containers of melted agar can be held in a 60 C water bath until needed.
6. Tape the ends of the gel form, if needed, and position in the comb.
7. Pour the agar into the gel form; it should come 1/3-1/2 of the way up the comb teeth.
8. Let the gel sit undisturbed until cool and firm (around 15 minutes). The agar will change in color from clear to slightly milky.

TIP: Gels can be prepared and poured several days before use. After cooling, wrap each gel in plastic wrap. Store gels at room temperature overnight or in the refrigerator for days.
TIP: Gels can be reused. Cut out the colored part of the gel and dispose of it. Store the rest in a sealed container in the refrigerator until needed. Re-melt the agar and pour it into gel forms.
TIP: If you have a choice of placing the comb in the middle of the gel, you may choose to have students deduce the charge of the molecules from their migration during electrophoresis.

SALT SOLUTION

Make enough to fill the electrophoresis chamber and cover the gel with 2-3 mm of liquid. Gel boxes made at our biotechnology workshops hold around 300 mls.

Method 1:
0.05 gram table salt (Sodium chloride)
1 liter tap water
Stir or swirl to dissolve.

Method 2:
1 gram table salt (Sodium chloride)
100 ml tap water
Stir or swirl to dissolve. Take 10 ml of this solution and add it to 990 ml of tap water. (You now have the same concentration as in Method 1.)

Method 3:
Add a small pinch of table salt to 1 liter of tap water. Stir or swirl to dissolve. Place this solution in the electrophoresis chamber and turn on the power. If small bubbles begin to rise through the solution from the electrodes, there is enough salt in the solution.

SAMPLES FOR ELECTROPHORESIS

Scientific stains: These stains produce discrete bands of color during electrophoresis. Many of the stains are negatively-charged (migrate toward the positive electrode), while a few are positively-charged (migrate toward the negative electrode). Most of the stains are not useful for creating "mystery" samples since they migrate approximately the same distance, even though they are different colors. Two stains, Xylene cyanol and Bromophenol blue, are commonly used as tracking dyes in DNA electrophoresis and separate well; Bromophenol blue migrates further than xylene

cyanol. Orange G migrates even further than either of these two stains and can be combined with them.

Preparing scientific stains

0.025 g powdered stain
10 ml deionized or distilled water
1 ml glycerol
Combine and mix thoroughly. Some stains may take a while to dissolve. Stains are best kept in brown or foil-covered bottles. Stains should not deteriorate over time.

LOADING GELS

The following gel-loading devices or methods will all work reasonably well. Load approximately 5µl/well.

Micropipetter tips and rubber Pasteur pipet bulbs. Place the micropipet tip in the bottom of the sample container without squeezing the bulb. Let the pipet fill by capillary action, remove it from the sample, and squeeze the bulb to load the sample in the gel.

Glass Pasteur pipets and rubber bulbs. Load one small drop/well.

Gel-loading Tips

Placing the gel form over a dark surface aids in visualizing the wells in the gel.
Use a clean micropipet tip or Pasteur pipet for each sample; do not mix samples.
If the wells in your gel are small and close together, only load samples in every other well. This will help reduce color mixing between wells and lead to better resolution of the bands.[1]
Use both hands when loading the gel; hold the sample-loading device in one hand and use your other hand and arm to support and stabilize the one doing the loading.
When loading samples into the gel, be careful to not puncture the bottom of the well; puncturing the well will allow the samples to mix.
If you have difficulty with food colors flowing between wells, try this technique:
Load the sample into the micropipet or Pasteur pipet.
Blot the tip on a paper towel.
Dispense the sample into the well in the gel.

RUNNING THE GEL

I suggest running the gel at 100 volts. Running the gel at higher voltages (up to 180 volts) will separate the dyes more quickly. However, more condensation will form on the lid of the chamber, making it difficult to watch the process. Higher voltages will also heat the gel, which may begin to dissolve.

If you are using a clear Plexiglass® electrophoresis chamber, you can place it on the glass of an overhead projector, allowing all of the students in your class to observe the separation and migration of the samples.

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*Negatively-charged stains: Acid fuchsin, Bromocresol green, Bromocresol purple, Bromophenol blue, Bromophenol red, Carbol fuchsin, Bromothymol blue, Eosin Y, Fast green FCF, Metanil yellow (acid yellow 36), Orange G, Phenol red, Xylene cyanol. Positively-charged stains: Basic fuchsin, Methyl green, Safranin O. Stains that do not move: Cresol red, Methylene blue, Neutral red, Toluidine blue, Cresyl violet.

The sample colors on the gel are best viewed against a white background, on a lightbox, or on an overhead projector.
Placing the gel on a piece of plastic wrap on the glass of an overhead projector allows all students to view it as they record and evaluate the results.
When the electrophoresis is completed and the power is turned off, the electrical gradient is removed and the dyes will begin to diffuse into the gel after a short period of time.
If you need or want to save the gels after electrophoresis, they can be wrapped in plastic wrap or put in a plastic bag and placed in the freezer. This stops the diffusion of the colors into the gel and is particularly helpful if your class is interrupted by a fire alarm or other unexpected event.