

ELECTROPHORESIS TEACHERS GUIDE

MAKING AGAROSE AND CASTING GELS:

Usually we use the lowest concentration of agarose possible since agarose is very expensive. For this activity we use 0.8% agarose by weight (i.e. for 100 ml of agarose, you would need 100 ml **buffer—not water** and 0.8g agarose powder). The percentage of agarose may be changed, depending on the size of the molecules or DNA fragments that will be separated. A higher agarose concentration makes a tighter gel which will allow only small pieces of DNA to fit through. This is the case in the PCR labs where we use 2% agarose gels, for separation of pieces of DNA by differences of 100 base pairs or less.

You can have students make their own gels, but if you are doing the activity in multiple classes, or pressed for time, it is much easier to make them yourself and store them (in a weigh boat) in baggies with a small amount of buffer so they don't dry out. Refrigeration is best to retard mold if you are not using the gels in two or three days. Just remember that the gels are rather delicate (especially at 0.8%) and will break if mishandled (see remelting below, however).

You can also prepare the melted agarose in a flask or bottle, allow it to cool, and cover and store at room temperature until ready to use. Agarose can be remelted (easiest in a microwave, but a hot water bath will do) and reused if it is relatively clean. You can run the dyes off the gel and remelt (but remember that the concentration of the gel will change—not a problem for the electrophoresis of dyes).

Buffer:

We use TBE Buffer which comes in 10x concentration. We use it at either 1x or .5x—as long as you are consistent, it really doesn't matter which concentration you choose. Remember that the buffer is reusable (at least for a few times), so you can use the same buffer for several classes in a row. Each gel box uses about 500ml of buffer, and each gel will need about 35 ml, so you will need to calculate how much buffer you will need first.

Let's say you need 8 gels and buffer for 8 boxes.

So you will need $8 \times 500\text{ml} + 8 \times 35\text{ml} = 4280$ ml of buffer needed (it's easier if you round this up to 4500 or even 5000) You can store the diluted buffer in bottles for the next year.

So for 4500 ml of 1X buffer. How much 10X buffer do you need?

If you use the formula $\text{Concentration}_1 \times \text{Volume}_1 = \text{Concentration}_2 \times \text{Volume}_2$

$$(C_1V_1 = C_2V_2)$$

$C_1 = 10x$, $C_2 = 1X$, $V_2 = 4500\text{ml}$ then

$$10x (V_1) = 1X (4500\text{ml})$$

$V_1 = 450$ ml of 10X Buffer and then add enough distilled water to make 4500 ml.

Materials:

Flask or bottle	gel casting tray
agarose powder	masking tape
1X TBE or TAE buffer	gel comb
hot plate or microwave	pot holder/hot hands/oven mitt

Procedure for making gels:

1. Calculate the amount of **buffer—not water** you will need for the number of gels you need to make. The large Stratagene gels take about 90 ml each and can be used for 2-3 groups each. The mini BioRad gels take about 35-40 ml each and used for single groups.
2. Place the buffer in a flask or bottle that will allow you to swirl the hot agarose without spilling it. Calculate the amount of agarose needed. Weigh and add to the bottle/flask. If the powder sticks to the sides, swirl to get it down into the solution. It is NOT easier to add the agarose first, since it tends to clump at the bottom and not mix well.
3. Heat the agarose until it begins to boil. **CAUTION: AGAROSE BOILS OVER EASILY!!!** It is easiest to use a microwave and heat for 30 seconds at a time, stopping to swirl the contents each time. If using a hot plate, be sure to watch CAREFULLY and stir constantly so the gel doesn't burn on the bottom. You should see that the solution is CLEAR. It may have a few small bubbles which will rise to the top as it cools.
4. As the agarose is cooling to a temperature where you can hold it without wincing, prepare the gel casting trays. All trays for this activity need the combs placed in the middle of the tray (so molecules can travel in both directions). For all other uses, combs are placed at one end of the gel tray.
5. Using masking tape,(or the gel clamp, or casting pieces—depending on the type of gel box you have) close off the ends of the tray. BE SURE THAT THE MASKING TAPE IS PRESSED FIRMLY ONTO THE ENDS OF THE TRAY. It's really ugly if it spills.
6. Pour the warm (not boiling hot) agarose slowly into the gel tray. The idea is not to have any bubbles, and to have an evenly cast gel. Fill to 2/3 to 3/4 of the casting tray.
HINT: Don't pour the last few drops—this is what causes bubbles. Large bubbles can be removed with a pipette as long as the gel is still liquid.
7. Leave the gel to cool and solidify (15-20 minutes). It will look somewhat cloudy and opaque. Store as needed.

OTHER PREPARATIONS:**Materials for Lab setup**

gel boxes	pipet tips
agarose gel	buffer solution
power supply	dye set (determined by teacher)
p20 micropipets	waste container
microcentrifuge	funnel for reclaiming buffer

1. Remember you need enough buffer for making the gels AND for filling the gel boxes, so be sure to calculate how much will be needed. Usually buffer is used at 1X concentration, but TBE can be used at 0.5 as well.
2. Buffer can be reused for some time. Used buffer can be stored in a carboy or bottle at room temperature. Pour buffer directly from the gel boxes back into the container. If you are using buffer that will go to another teacher in the group, please do not mix used with unused buffer.
3. Dyes are made up in the kits. The following are the dyes included by number:
 1. Xylene cyanol (negative dye)
 2. Phenol red (negative dye)
 3. Bromphenol blue (negative dye)If you need to make dyes (for 100 ml):

4. Orange G	(negative dye)	89ml distilled water
5. Methylene blue	(positive dye)	10ml glycerol
6. Congo red	(negative dye)	1 ml 1M TRIS pH8
7. Bromcresol green	(negative dye)	.2ml 0.5M EDTA pH8
8. Brilliant blue	(negative dye)	.25 g dye
9. Brilliant green	(positive dye)	
10. Methyl green	(positive dye)	
11. Bromcresol purple	(negative dye)	

You can assign dyes to lab groups, allow them to choose, or a combination of both. For an “unknown” see #4 below.

4. Make a combination of dyes from the list and see if students can figure out which dyes may be included in the mix. If you do this, be sure to assign the dyes used in the mix for running on the gel, along with others not in the mix.

RUNNING THE GELS: Gels should be run at 100-150 volts for 15-20 minutes. You should stop the gels after about 15 minutes by turning off the power, and removing the top. Check the dyes to see if you are getting enough separation to stop at that point. If not, reconnect the top and turn on the power for another 5 minutes. Repeat as necessary.

ELECTROPHORESIS TEACHING TIPS:

Since this is the first time students will be using the gel boxes, modeling the procedure is important. You should also check every team before they plug in the boxes to be sure that the gel is covered in buffer. Also check to be sure that the gel is not floating—it should be pressed down into the gel tray.

The trickiest part is the loading. You may want to make up some practice gels, and allow students to try loading these with food coloring solution before proceeding with this lab. The dyes are mixed with glycerol which is denser than the buffer and will fall down into the well with the dye. Gels can be made with multiple combs, or made in a petrie dish (just let the combs rest on the sides) for practice. If you want to save money, you can do this with gelatin.

Students need to be reminded about which stop on the pipet is for drawing up liquid and which is for expelling. Students who don't know the difference will be sucking the dyes back up rather than loading them onto the gel.

Usually when loading gels, we leave the outermost lanes (wells) empty. They tend to bend and not give good results, but can be used when space is a problem. This is NOT a problem with the dyes, but it is something to be aware of later.

Recording the results of the electrophoresis **MUST** be done on the same day the gels are run. The dyes (and anything else you run) will diffuse away if it is left for any length of time.

Dyes stain hands and clothes.

Agarose gels can be disposed of directly into the garbage. **BE CAREFUL NOT TO PUT AGAROSE INTO THE SINK!!!!**