

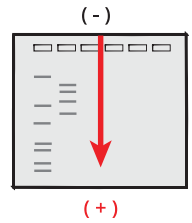
QUICK GUIDE: Electrophoresis

WHAT IS ELECTROPHORESIS?

Electrophoresis is a technique that allows us to separate DNA, RNA or proteins according to their size. DNA electrophoresis is commonly done using an agarose gel of a varying percent concentration based on the size of the DNA bands. Agarose gels are porous and allow the DNA to migrate through the gel, leaving behind separated bands based on the number of base pairs. The buffer that is used to make the gel is the same buffer that has to be used to run the gel (for example, a TAE gel needs to run in a TAE buffer). The buffer maintains the pH of the experiment and also supplies the ions needed for the migration of the bands!

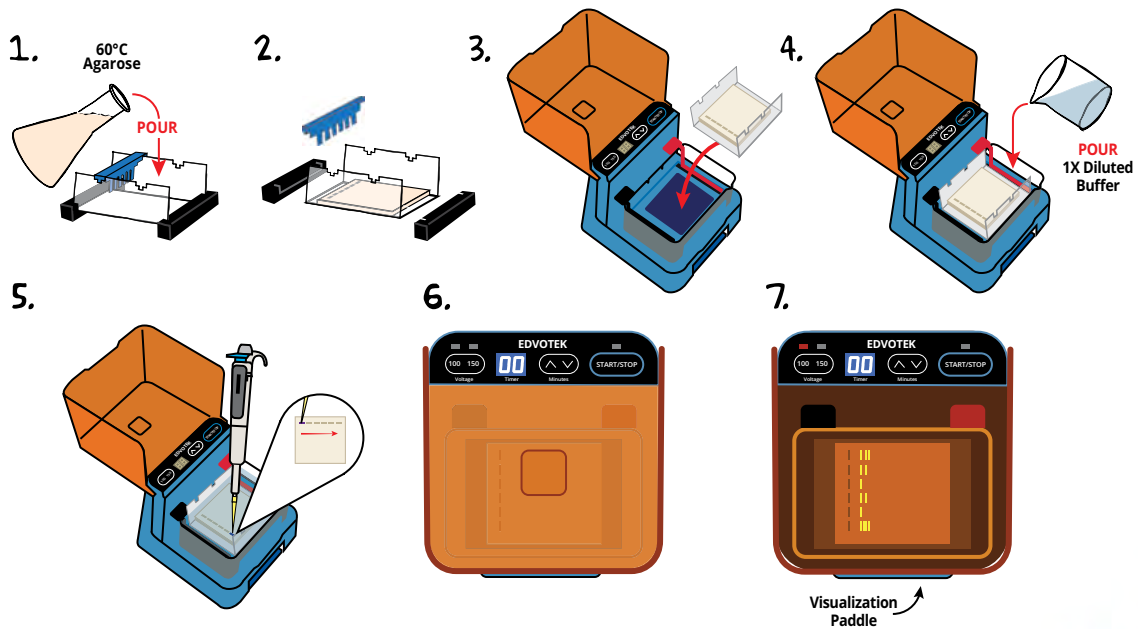
HOW DOES ELECTROPHORESIS SEPARATE DNA FRAGMENTS?

The mixture of DNA molecules is added into depressions (or “wells”) within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode.



WHAT IS THE EDGE™, AND HOW IS IT DIFFERENT FROM OTHER ELECTROPHORESIS SETUPS?

The EDGE™ (Cat. #500) is Edvotek's integrated electrophoresis apparatus. It offers all you need for electrophoresis in one unit: the electrophoresis apparatus, power supply, and visualization with a blue light transilluminator. Other electrophoresis set ups require separate pieces of equipment for running, powering, and viewing your experiment.



RELATED VIDEO:
Performing Electrophoresis
Using the EDGE™!



AGAROSE GEL ELECTROPHORESIS TIPS AND TRICKS:

While the EDGE™ saves time when completing an experiment (if you stain with SYBR® Safe, you can visualize banding within 10 minutes), there are other tips and tricks you can use to help optimize your electrophoresis experiments.

• **Prepping gels ahead of time:**

Are your lab periods busy and you just need more time? Try prepping your agarose gels ahead of time! Simply make the gels, de-cast them, and store them in a zip-top bag or plastic wrap with about 2 mLs of buffer to keep them moist. Then just stick them into the refrigerator. This can be done up to a week ahead of your experiment time.

• **Make sure your buffer is diluted correctly:**

In our EdvoKits, we send out a 50X Concentrate of TAE buffer, or powdered TBE buffer. It is important to follow the instructions in the instructor guide to dilute your buffer to the 1X concentration it needs to be to run! If it isn't diluted properly, you run the risk of your buffer overheating, the gel melting, and the experiment not working.

Table D Diluting Electrophoresis Buffer		
Amount of 50X TAE	Amount of Distilled Water	Final Volume
20 mL	980 mL	1000 mL
40 mL	1960 mL	2000 mL
60 mL	2940 mL	3000 mL

Example directions for diluting electrophoresis buffer.

• **Chamber breaks to reduce overheating:**

If you are going to use the same electrophoresis chamber for multiple runs, make sure you either replace the buffer every other run, or let the chamber cool down before starting your next gel.

• **Let your gel cool completely before using it:**

If you don't allow the agarose gel to completely solidify, the heat from the buffer may cause it to melt or distort the bands. The best rule of thumb is to let the gel sit for 15-30 minutes before using it.

• **Practice makes perfect:**

Use the provided practice gel loading solution to practice loading into the wells. This can be done using the actual gel, or one of the DuraGels™ (Cat. #S-43). Make sure that you don't accidentally puncture the bottom of the well, rest the pipette against the well wall, or overload the gel as these will all alter the quality of the results.

SYBR® SAFE DILUTION:

SYBR® Safe (Cat. #608) comes as a concentrate so it is very important to dilute it according to the directions in your experiment literature. Since it is so concentrated, we only send out a few microliters, so it is important to centrifuge the tube before opening so you don't accidentally spill what is in the tube! Once the SYBR® Safe is diluted, it can be added to the molten agarose in a 1:1 ratio, meaning for every mL of agarose you will add 1 µL of diluted SYBR® Safe.

Prepare SYBR® Safe Stain:

1. Following the instructions in Appendix B, prepare 1x Electrophoresis Buffer by combining 10 µL of 50X Concentrated Buffer with 490 µL of distilled water.
2. Add 390 µL of the 1X buffer from step 1 to the tube of SYBR® Safe and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

Example directions for diluting SYBR® Safe Stain.

CHECK OUT OUR RELATED YOUTUBE VIDEOS:



Diluting SYBR® Safe DNA Stain



Preparing an Agarose Gel For Electrophoresis



Principles and Practice of Agarose Gel Electrophoresis

