EDVOTEK® QUICK GUIDE

Agarose Gel Electrophoresis

WHAT IS ELECTROPHORESIS?

Electrophoresis is a technique that allows us to separate DNA, RNA or proteins according to their size.

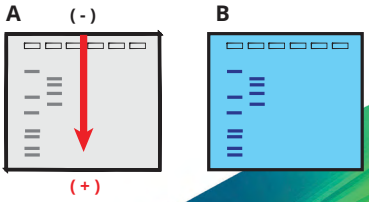
WHAT DO I NEED TO SEPARATE A MIXTURE OF DNA MOLECULES?

In addition to your DNA sample, you will need:

- Gel Loading Solution – includes glycerol to help DNA samples enter into the wells and a visible dye to monitor migration through the gel.
- Agarose – a polysaccharide used as the separation matrix.
- Electrophoresis Buffer – contains ions necessary to conduct an electrical current, maintains pH of experiment.
- Horizontal electrophoresis apparatus – holds the buffer and the gel, has positive and negative electrodes.
- Power supply – generates the current necessary to move DNA through gel.
- Micropipette – used to transfer samples into wells.
- A special stain that allows us to visualize DNA.

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 (Figure A).



At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure B).

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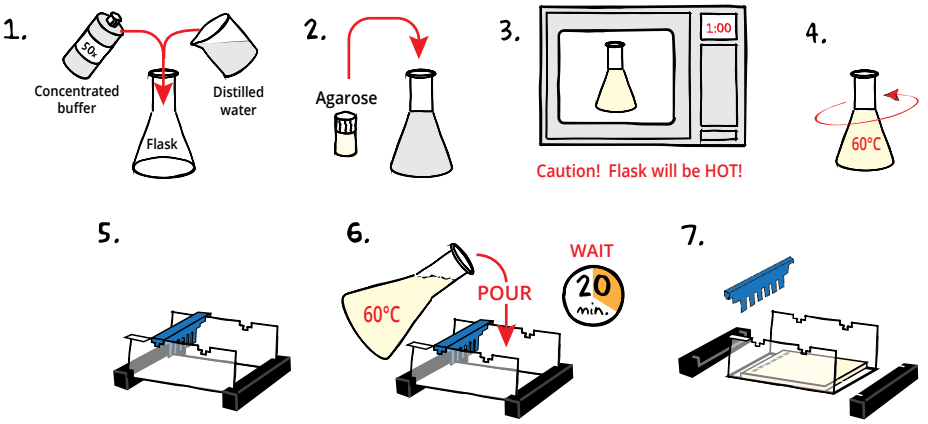
Quick Guide: Agarose Gel Electrophoresis



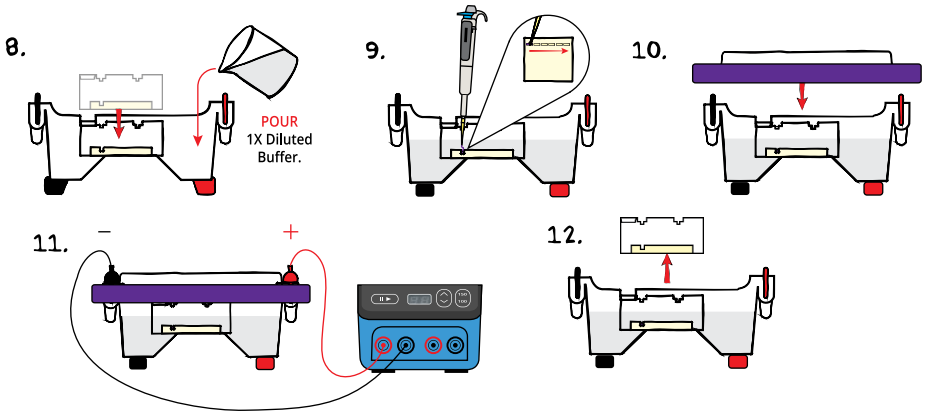
CASTING THE AGAROSE GEL

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and MIX by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*



RUNNING THE GEL

8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** the entire sample into the well in the order indicated by your experiment.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and PERFORM electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

PROCEED to staining and visualizing agarose gels using FlashBlue™ Stain.

**Gels that have previously been removed from their trays should be "anchored" back to the tray with a few drops of molten agarose before placing into the electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.*

Table B

1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C

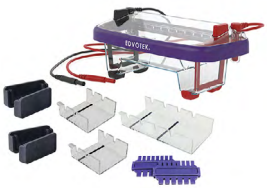
Time and Voltage Guidelines
(0.8% Agarose Gel)

	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

Electrophoresis Equipment

Visit our website for our full range of electrophoresis and power supplies at:
www.edvotek.com

Cat. #500
EDGE™ Integrated Electrophoresis System



Cat. #502-504
M12 Complete™ Electrophoresis Package

Cat. #515
M36 HexaGel™ DNA Electrophoresis Apparatus

Cat. #589 - #593
EDVOTEK® Variable Micropipettes
From 0.1 µL to 5000 µL

Cat. #509
DuoSource™ Power Source
75 or 150 volts

Cat. #5010-Q
QuadraSource™ Power Source
10-300 volts

Cat. #585 - #588
EDVOTEK® Fixed Volume Minipipettes
From 5 µL to 200 µL

Cat. #557
TruBlu™ 2 Blue/White LED Transilluminator
27 x 15 cm viewing surface

Cat. #558
Midrange UV Transilluminator
7 x 14 cm UV filter

Cat. #540
EdvoCycler™ Jr. Personal PCR Machine
Holds 16 x 0.2 mL tubes

Cat. #541-542
EdvoCycler™ 2
Holds 48 x 0.2 mL tubes