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.
- Micropipet – 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 (Fig. 1A). Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Fig 1B).

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 1C).

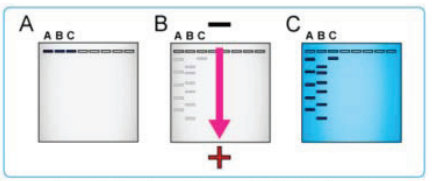

Figure 1: Overview of Agarose Gel Electrophoresis

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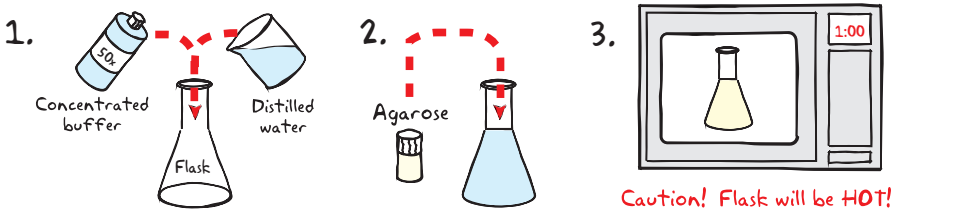

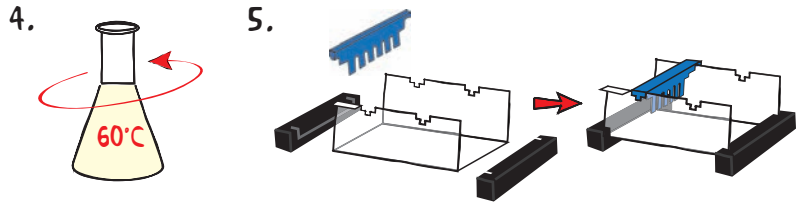

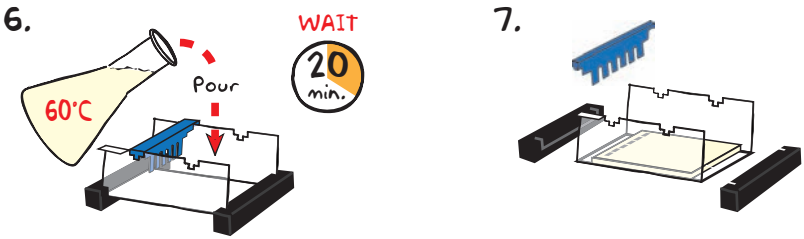

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask 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™ Gel

DNA Staining with FlashBlue™ or InstaStain® Blue

Size of Gel Casting tray	Amt of Agarose	+ Concentrated Buffer (50x)	+ Distilled Water	= TOTAL Volume
7 x 7 cm	0.23 g	0.6 ml	29.4 ml	30 ml
7 x 10 cm	0.39 g	1.0 ml	49.0 ml	50 ml
7 x 14 cm	0.46 g	1.2 ml	58.8 ml	60 ml

Wear Gloves and Goggles

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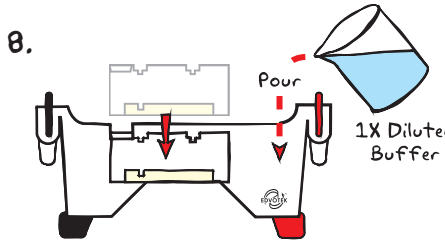

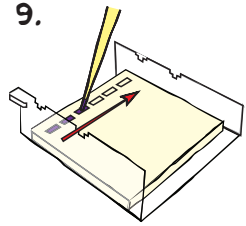

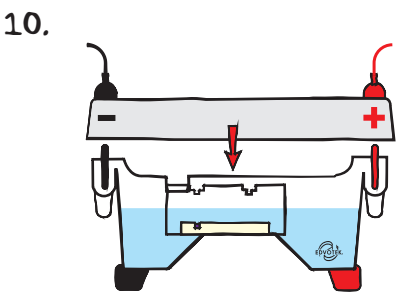

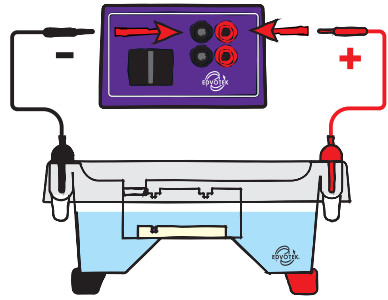

8. **PLACE** gel (on the tray) into electrophoresis chamber. Completely **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes).
9. **LOAD** 25 µl of the DNA samples into wells in consecutive order.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA 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).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING & VISUALIZATION.**

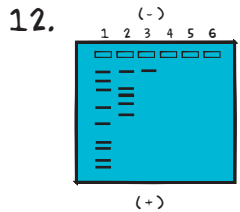

Table B — 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 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: M6+	M12 & M36
Volts	Min. / Max.	Min. / Max.
150	10 / 15 min.	20 / 30 min.
125	15 / 20 min.	35 / 45 min.
70	35 / 45 min.	60 / 90 min.
50	50 / 80 min.	95 / 130 min.

Electrophoresis Equipment

See the **EQUIPMENT** section in our Resource Guide for our full range of electrophoresis and power supplies or visit our website at:
www.edvotek.com

Cat. #515
M36 HexaGel™ DNA Electrophoresis Apparatus
For 6 Lab Groups

Cat. #502/504
M12 or M12 Dual Electrophoresis Apparatus
For 2 Lab Groups

Cat. #500
M6Plus Electrophoresis Apparatus
For 1 Lab Group

Cat. #5010
TetraSource™ 300 Power Supply
30-300 V for 1 to 4 units

Cat. #509
DuoSource™ 150
75/150 V for 1 or 2 units

Cat. #507
DuoSource™
75 V for 1 or 2 units

Cat. #589-#593
EDVOTEK® Variable Micropipets
From 0.1 µl to 5000 µl

Cat. #585-#588
EDVOTEK® Fixed Volume MiniPipets™
From 5 µl to 200 µl

Cat. #541
EdvoCycler™
Holds 25 x 0.2 ml tubes

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