



PURPOSE

This laboratory explores the principles of electrophoresis, a powerful technique used in biomedical research and biotechnology.

BACKGROUND

Electrophoresis is a technique developed in the 1930s for separating and analyzing charged molecules. In fact, the term *electrophoresis* literally means "to carry with electricity."

The three main parts of an electrophoresis system are

- ◆ a **power supply**, which converts alternating current electricity (AC, from the wall outlet) into direct current electricity (DC), and which allows voltage to be set.
- ◆ a plastic electrophoresis apparatus (**gel box**).
- ◆ a solution of water and ions that conducts current. Ions are atoms (or groups of atoms) that have lost or gained electrons and can conduct electrical current.

Look inside the gel box and find a metal strip or wire at each end. These are called electrodes.

- ◆ The electrode at which electrons enter the gel box from the power supply (along the black wire) is called the **cathode** and is negative (-).
- ◆ The electrode at which electrons leave the box and re-enter the power supply (along the red wire) is called the **anode** and carries a positive charge (+).
- ◆ Electron flow from the power supply sets up a potential energy difference between the electrodes. This energy difference is known as **potential**, is measured in **volts**, and establishes an electric field through which the ions in the gel box solution migrate.
- ◆ The migration of ions in the fluid creates electrical **current** which is measured in milliamperes (**milliamps** or mA).

MATERIALS PER TEAM

distilled water	150 ml beaker
NaCl solution (5M)	P1000 micropipet & tips (shared)
TAE buffer (1X)	plastic transfer pipet (to measure phenol red)
phenol red solution (saturated)	plastic transfer pipet (to use as stirrer)
pH paper	protocol card (<i>Gel Box/Power Supply</i>)
gel box	container for waste
power supply (DC), shared	

CAUTION

- ◆ Study the gel box and its *safety interlock lid*. It is designed so that electrical contact must be disabled (i.e. electrical leads disconnected) before raising the lid.
- ◆ Since any wet surface can become conductive, it is advisable **NOT** to **touch** any part of the apparatus (gel box, wires) while the power supply is on. This is especially important if the outside of the box is wet or if your hands are wet.



PROCEDURE

As you work through the following steps, observe what is happening inside the gel box.

Complete the Electrophoresis Exploration Record Sheet while performing the lab.

1. Set the gel box in front of you; the "front" is the side on which the logos are displayed (e.g. BRL, Horizon 58). Position the box near a power supply, but do NOT connect it yet.
2. Examine the **power supply**, identify the following, and write the names in the blank spaces on page 1 of the Record Sheet. Refer to the protocol card *Gel Box/Power Supply*.
 - check fuse light
 - digital meter
 - power switch (on/off)
 - voltage range switch (low/high)
 - two sets of DC output terminals
 - meter function switch (V/mA)
 - current overload light
 - voltage select knobExamine the **gel box** and identify the following; write the names in the spaces on page 1 of the Record Sheet.
 - safety interlock lid
 - anode
 - cathodeDraw lines (in red and black, if possible) representing the electrical leads (sometimes called power cords) to show how both gel boxes A & B should be connected to the power supply at the same time.
3. Remove the plastic gel deck from the gel box. With the power supply OFF and the safety lid down, connect the empty gel box to the power supply with the electrical leads ("red to red, black to black"). Connect gently by pushing straight in; there is no need to twist the connectors!
4. Set the voltage range switch to LOW, turn the power supply ON, and select a potential of about 100 V (it need not be exact). Put the meter function switch on milliamps and note (on the Record Sheet, page two, step 4) the current generated in the **empty gel box**.
5. Turn the power supply OFF and disconnect the electrical leads.
6. Lift the safety lid, and add about 125 ml of **distilled water**. Lower the lid, reconnect the electrical leads, and turn the power supply ON. Turn the voltage selector to get 100 V. Record the current in mA on the record sheet.
7. Turn the power supply OFF and disconnect the electrical leads.
8. Lift the lid and add 250 μ l of 5M sodium chloride (NaCl) solution to the distilled water in the box. Carefully mix the contents of the box with a stirring rod (or transfer pipet). Lower the lid, reconnect the electrical leads, and turn the power supply ON. Select approximately 100 V. Record the current in milliamps. Now connect *two* gel boxes containing sodium chloride solution to the same power supply. Record the current.

****WHEN TWO BOXES ARE CONNECTED TO ONE POWER SUPPLY, DIVIDE THE CURRENT SHOWN IN HALF TO GET MILLIAMPS PER BOX.****

9. Record the current at the following voltages on the Record Sheet:
 - a. 25 V
 - b. any voltage between 25 and 100.
 - c. 100 V
 - d. Move the voltage range switch to "HIGH" and select any voltage between 100 and 250 V
 - e. 250 V

When current is flowing, chemical reactions occur at the cathode and at the anode

At the cathode: 4 electrons⁻ (from power supply) + 4 H₂O → 2 H₂ (hydrogen gas) + 4 OH⁻

At the anode: 4 H₂O → O₂ (oxygen gas) + 4 H⁺ + 2 H₂O + 4 electrons⁻ (back to power supply)

The equations indicate that gases will be produced at the electrodes. How do you know? What do you see?

The equations also indicate that the solution around one electrode will become acidic (H⁺) and the solution around the other will become basic (OH⁻). Do you see the hydroxide ions (OH⁻)? Do you see the hydrogen ions (H⁺)? Are corresponding pH differences developing in the gel box solution? Let's find out!

10. Turn the power supply OFF and disconnect the electrical leads.
11. Lift the lid and thoroughly stir (at least 10 times), mixing the gel box solution. Record the pH at both ends of the gel box with pH strips.
 - Take one pH strip. Hold it by the arrow section only.
 - Carefully dip the entire strip into the solution to be measured, remove the strip from the solution. Compare the color of the center section to the numbered colors on the strip.
 - The pH of the solution is the number with the matching color.
 Record the pH results in the observation column for Step 11.
12. Using a transfer pipet, add 1 ml of saturated phenol red (an indicator) to the salt water in the gel box and stir. Keep stirring until the solution in the gel box reaches a uniform color.
13. Lower the lid, reconnect the electrical leads, and turn the power supply ON. At 100 V, record the current. Notice any color changes that develop within 3-5 minutes observation and record in the observation column for Step 13/14.
14. Without jostling the box or mixing the solution, turn the power supply OFF and disconnect the electrical leads. Raise the lid and record the pH at both ends of the gel box. Record with the observations for Step 13/14.
15. Discard the sodium chloride solution. Obtain 125 ml 1X TAE buffer. Add 1 ml saturated phenol red to the 1X TAE in your beaker and mix well so that the color is uniform. Measure and record the pH of the buffer and pour the buffer into the gel box.
16. Lower the lid, reconnect the electrical leads, turn the power supply ON again and select about 100 V. Observe color changes that develop within 3-5 minutes of observation. Record the pH at both ends of the gel box.

Upon completion of this lab

- ◆ Dispose of designated materials in the appropriate places.
 - Lift the plastic buffer tray out of the apparatus and pour its fluid down the sink.
 - Rinse with distilled water and pat dry the outside of the tray. Put the tray back into the gel box. Place the gel deck back in the gel box.
- ◆ Leave equipment as you found it; check that your work station is in order.
- ◆ Wash your hands.