EmbiTec Mini-One Agarose Gel Electrophoresis (uses Gel GREEN)

Amgen Biotech Experience
Scientific Discovery for the Classroom
Greater Los Angeles

Gel Green is non-toxic, light sensitive and must be stored at Room Temperature.
Gel green can be visualized with the uV transilluminator and with the blue transilluminator.

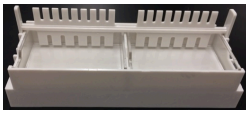
Make 1 liter 1X Sodium Borate buffer:

- Measure 50 mL 20X SB in graduated cylinder. Pour into bottle.
- Measure 950 mL dI-H_20 in graduated cylinder. Add to bottle, cap and mix.

Make/melt 100 mL 0.8% Agarose Gel:

- Measure 100 mL of 1X SB buffer in graduated cylinder.
- Pour some of the buffer into E-flask (or container).
- Weigh out 0.8 grams of dry agarose with weigh boat on scale and add to the buffer in flask.
- Use rest of buffer to wash down all of the agarose (if needed).
- Cover with **vented** cap (or very loose solid cap).
- Microwave on High until bubbling starts (about 1 minute). Use gloves to swirl flask.
- Reheat until bubbling again (about 15 seconds). Use "hot hands" or gloves to swirl flask.
- See if the solution clear and has no specks. Reheat if needed.
- Set aside to cool to 65-70° C (when you can hold the flask with bare hand).

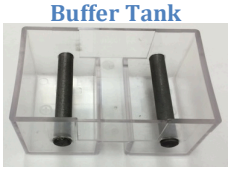
- Keep melted agarose (capped) in a 65° C water bath, if you cannot pour gels right away.
- If you want students to pour the gels, transfer 20 mL into conical tubes. Cap tightly and place in 65° C waterbath.



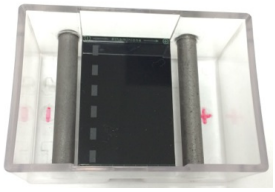
Pour Agarose gel with EmbiTec Mini One System

- Place the two agarose gel trays into the casting stand.
- Insert the comb (usually the larger teeth down) into the casting stand.
- Pour about 10 mL agarose solution (around 65°C) into each gel tray. Agarose should reach about halfway up the comb teeth.
- Do not worry about the little bit of gel solution that flows underneath the trays.
- Check and remove any air bubbles with a pipet tip.
- Do not move the casting stand for about 20-30 minutes, or until gel turns opaque.
- Drip 1X buffer around the comb. Remove comb by gently pulling upwards.
- Gently squeeze tray sides and pull the gel trays up to remove from the casting stand. You can scrape the bottom of tray along the edge of the stand to remove excess gel.
- Be sure to keep gel tray horizontal, so that the gel does not slide off the tray.
- You can make gels ahead of time, place into plastic box with a little bit of buffer into the wells, cover tightly, store at RT. If you do not store gel with tray, then you will need to transfer the gel back onto the tray before placing into electrophoresis chamber. Be sure that the gel is not flipped upside down onto the tray.
- Leave extra agarose in the flask and mark what % agarose it is).
- Rinse casting stand and comb with water.

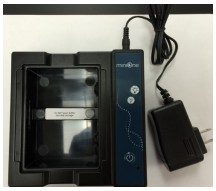
Buffer Tank



Set up the EmbiTec Mini One Gel Electrophoresis System



- See our video at http://youtu.be/KDC0tlAZH9k
- Be sure the outside of the clear buffer tank is dry, especially at the two electrode connections (bumps on the outside).
- If needed, place the black plate onto the middle platform of buffer tank. The buffer tank with black plate should now look like this, with the wells near the negative side.



- The black carriage has a built-in power supply and blue transilluminator.
- Insert the clear buffer tank into the black carriage. It only fits one way. Be sure that it is seated all the way down, so that the electrodes make the connection.
- Place the casting tray (with a solidified agarose gel) on top of the black plate in the buffer tank in the correct orientation.
- Pour 1X buffer to one sides of the buffer tank, so that it covers the gel (about 125 mL). Do not use too much buffer.

SAMPLE PREPATION for EmbiTec Mini One Gel Electrophoresis System

- Use Loading Dye that contains **GEL GREEN** for this system. Gel Red will not work!
- Store the GGLD (Gel Green Loading Dye) tube at **Room Temperature in the dark** (the provided amber tube will protect against light). If you store the GGLD in the refrigerator, the dye will precipitate and DNA staining may be very faint or nonexistent.
- If you aliquot GGLD to give to your students but have to use clear microfuge tubes, cover the entire tube rack with a box or foil, and pass out the GGLD just before use.
- Students will need to add 2 uL GGLD to each of their 10 uL DNA samples. Mix.
- Teacher will need to add 2 uL GGLD for every 10 uL DNA ladder marker. Mix.
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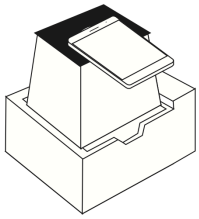
- Plug in the power cable.
- Turn on the low intensity light to help see the wells. Load samples into gel wells.



Run the Embitec Mini One Gel Electrophoresis System

- The hood has a built-in filter for the blue transilluminator, so that cell phone photos can be easily taken. Suggest students take photos every 5 minutes of their 15-20 minute run.
- The hood is absolutely **required** on top of the black carriage for the power to stay on.
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- Do not bump the carriage. Place hood on top. Turn power on (and a green LED will light up to show that power is on).
- At 5 minutes, turn on the light (two intensities available) to see the DNA bands enter gel.
- Watch the DNA bands move through the gel and take photos with cell phone or digital camera. Do not zoom in.
- You can stop the run at 15-20 minutes. You will not be able to see the complete separation of the DNA ladder, but you should be able to see the DNA bands from the restriction digest(s).

- **IMPORTANT:** If you run the gel for over 20 minutes, the small sized DNA band will fade from view around 20 minutes. Orange G dye will be at bottom of gel by 30 minutes.
- **Note:** If the hood is removed while the gel is running, the power will automatically shut off. Replace the hood and then TURN ON the power again to resume the run.

Clean Up the Embitec Mini One Gel Electrophoresis System

- You can dispose of the Gel Green gels in regular trash. Or you can still throw them into the biohazard waste bag if you prefer.
- Unplug the power supply. Remove the buffer tank from the black carriage/power supply. Empty the buffer tank into the sink. Rinse off the gel tray and buffer tank with WATER only (never use any alcohols or acetone). Invert and allow to air dry.
- NEVER get the black carriage/power supply wet.

Pack Up the Embitec Mini One Gel Electrophoresis System