Horizontal Electrophoresis

Protocol

Bulletin 6206

Agarose Gel Preparation for DNA Separation

Agarose gels can be used to separate DNA of various sizes. Before casting an agarose gel determine the appropriate percent agarose gel to use, based on the size of DNA to be separated.

Procedure

1. Determine the amount of agarose (grams) required to make the desired agarose gel concentration and volume. Use tables below as a guide for agarose concentration and gel volume requirements.

Example: For a 1% agarose gel, add 1 gram of agarose to 100 ml of 1× electrophoresis buffer.

Gel concentration required for DNA separation.

Gel Concentration (%)	DNA size
0.50	1–30 Kb
0.75	800–10 Kb
1.00	500 bp–10 Kb
1.25	400 bp–7 Kb
1.50	200 bp–3 Kb
2.00*	100 bp–2.5 bp
3.00*	40 bp–2Kb
4.00**	10–400 bp

* Sieving agarose such as Certified™ PCR agarose

** Sieving agarose such as Certified low range ultra agarose

Gel volume requirements.

Gel Size (thickness)	0.25 cm	0.5 cm	0.75 cm	1.0 cm
Base				
7 × 7 cm	10 ml	20 ml	30 ml	40 ml
15 × 7 cm	20 ml	40 ml	60 ml	80 ml
15 × 15 cm	50 ml	100 ml	150 ml	200 ml
25 × 10 cm		125 ml	185 ml	250 ml
25 × 15 cm		185 ml	280 ml	375 ml
Tray				
7 × 7 cm	10 ml	20 ml	30 ml	40 ml
7 × 10 cm	15 ml	30 ml	45 ml	60 ml
15 × 7 cm	20 ml	40 ml	60 ml	80 ml
15 × 10 cm	30 ml	60 ml	90 ml	120 ml
15 × 15 cm	50 ml	100 ml	150 ml	200 ml
15 × 20 cm	70 ml	140 ml	210 ml	280 ml
15 × 25 cm	90 ml	180 ml	270 ml	360 ml
25 × 10 cm		125 ml	185 ml	250 ml
25 × 15 cm		185 ml	280 ml	375 ml
25 × 20 cm		250 ml	375 ml	500 ml
25 × 25 cm		310 ml	465 ml	625 ml

2. Add the agarose to a suitable container (e.g., 250 ml Erlenmeyer flask, Wheaton bottle, etc.). Add the appropriate amount of 1× electrophoresis buffer and swirl to suspend the agarose powder in the buffer.

3. The agarose can be melted by boiling on a magnetic hot plate or in a microwave oven.

4a. **Magnetic hot plate method** — add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Bubbles or foam should disrupt before rising to the neck of the flask.

4b. **Microwave oven method** — place the gel solution into the microwave. Using a low to medium setting, set the timer for a minimum of 5 min, stopping the microwave oven every 30 sec and swirling the flask gently to suspend the undissolved agarose. This technique is the fastest and safest way to dissolve agarose. Boil and swirl the solution until all of the small translucent agarose particles are dissolved. With the small flask still in place, set aside to cool to 60°C before pouring.

Casting Agarose Gel Slabs

There are several ways to cast agarose submarine gels using the Sub-Cell® GT and Sub-Cell Model 96 and 192 systems. Gels may be cast with a UV-transparent plastic (UVTP) tray directly on the gel stage of the electrophoresis bases using the gel casting gates. Gels may also be cast on the removable UVTP trays with the aid of the gel caster or with standard laboratory tape.

Casting gels on the base stage with the UVTP tray

1. Level the cell using the leveling bubble provided.
2. Place the UVTP tray on the gel stage.

Note: The Mini-Sub® cell GT requires the 7 × 7 cm UVTP tray for casting in the GT base. The wide-Mini-Sub cell GT requires the 15 × 7 cm UVTP tray and the Sub-Cell GT system requires the 15 × 15 cm UVTP tray for casting in the GT base. Sub-Cell Model 96 system requires the 25 × 10 cm UVTP tray for casting in the base, Sub-Cell Model 192 system requires the 25 × 15 cm UVTP tray for casting in the base.

Insert the gel casting guide (table) from page 259 (2010/2011 catalog)

3. Slide the gel casting gates into the slots at opposite ends of the gel stage. Insure that the gates are evenly seated in the slots and the gates uniformly contact all edges of the UVTP tray. The weight of the gates provides a tight seal to prevent any leakage problems during gel casting*.

* **Note:** If leakage occurs while pouring the gel on the casting tray atop the stage, chill the casting gates in the freezer for 2–3 min. Place the casting gates into the slots when ready to pour the gel. The chilled casting gates will prevent the gel solution from leaking out of the tray and into the chambers.

4. Place the comb(s) into the appropriate slot(s) of the trays so that the sample wells are near the cathode (black). DNA samples will migrate toward the anode (red) during electrophoresis.
5. Prepare the desired concentration and amount of agarose in 1× electrophoresis buffer. When the agarose solution has cooled to 50–60°C, pour the molten agarose between the gates.

Warning: Hot agarose (>60°C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.

6. Allow 20–40 min for the gel to solidify at room temperature.
7. Carefully remove the comb from the solidified gel. Remove the gel casting gates.
8. Submerge the gel beneath 2 to 6 mm of 1× electrophoresis buffer. Use greater depth overlay (more buffer) with increasing voltages to prevent pH and heat effects.

Removable tray (UVTP) gel casting using a gel caster or mini-gel caster

1. Level the gel caster or mini-gel caster using the leveling feet in the gel caster and the leveling bubble provided.
2. Disengage and slide the movable wall to the open end of the gel caster or mini-gel caster by turning and lifting the cam peg upward.

Note: If casting more than one gel with the gel caster, add the removable gel casting wall to the gel caster. The removable wall will allow casting using two 15 × 10 cm trays, four 7 × 10 cm trays or one 15 × 10 cm and one 15 × 15 cm or larger trays.

3. Place the open edge of the UVTP tray against the fixed wall of the gel caster or mini-gel caster.
4. Slide the movable wall against the edge of the UVTP tray (Figure 1).

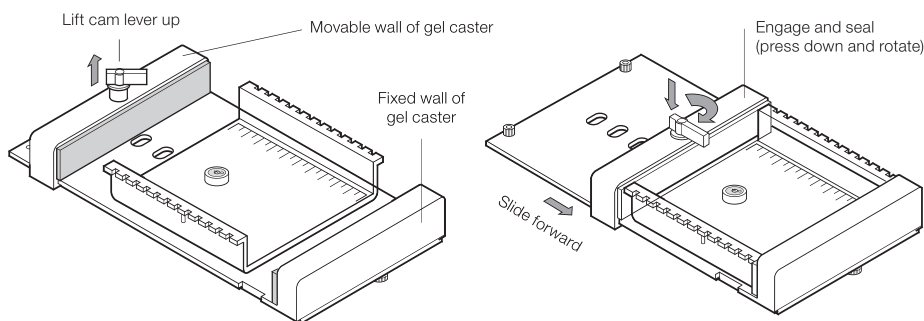

Fig. 1. Sealing the UVTP tray for gel casting.

5. To seal the open tray ends, engage the cam peg by turning and pressing downward simultaneously.
6. When the cam peg has dropped into the appropriate slot, turn the peg in either direction until resistance is felt. This action seals the edges of the tray for casting.
7. Place the comb(s) into the appropriate slot(s) of the tray.
8. Prepare the desired concentration and amount of agarose in 1× electrophoresis buffer. When the agarose solution has cooled to 50–60°C pour the molten agarose between the gates.

Warning: Hot agarose (>60°C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.

9. Allow 20–40 min for the gel to solidify at room temperature.
10. Carefully remove the comb from the solidified gel.
11. Disengage the cam peg by turning and lifting upward. Slide the movable wall away from the tray. Remove the tray from the gel caster or mini-gel caster.

Note: While the gel is solidifying, a light seal is formed between the gasket and the gel (especially for low percentage agarose gels [<0.8%]). Before moving the wall away from the tray, carefully lift the tray on one side to release the seal or use a spatula to break the seal between the agarose and gasket.

12. Place the tray onto the leveled electrophoresis base so that the sample wells are near the cathode (black). DNA samples will migrate toward the anode (red) during electrophoresis.
13. Submerge the gel beneath 2 to 6 mm of 1× electrophoresis buffer. Use greater depth overlay (more buffer) with increasing voltages to avoid pH and heat effects.

Removable tray (UVTP) gel casting using tape

1. Seal the ends of the UVTP gel tray securely with strips of standard laboratory tape. Press the tape firmly to the edges of the gel tray to form a fluid-tight seal.
2. Level the gel tray on a leveling table or workbench using the leveling bubble provided with the instrument.
3. Prepare the desired concentration and amount of agarose in 1× electrophoresis buffer. When the agarose solution has cooled to 50–60°C pour the molten agarose into the gel tray.

Warning: Hot agarose (>60°C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.

4. Allow 20–40 min for the gel to solidify at room temperature.
5. Carefully remove the comb from the solidified gel.
6. Remove the tape from the edges of the gel tray.
7. Place the tray onto the leveled Sub-Cell base so that the sample wells are near the cathode (black). DNA samples will migrate toward the anode (red) during electrophoresis.
8. Submerge the gel beneath 2 to 6 mm of 1× electrophoresis buffer. Use greater depth overlay (more buffer) with increasing voltages to avoid pH and heat effects.

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