**Western Gel Making Protocol**

**Making Gel Caster:**

* + - 1. Preparation of gel plates/Gel caster
         1. Put the glass plates and Aluminum backing plates [with a 0.75 mm spacer] on a clean surface [like a paper towel].
         2. Spray Clean with 100% methanol and Kim-wipe. [PS: they are expensive so don’t break.] – Clean – sweeky clean!!!
      2. Putting the gel plates on the gel caster unit to create the gel-plate sandwich
         1. Insert the spacers [black ones] on the sides of the plates [1 for each side, so 2 for 1 pair of plates]. This must be done carefully until the spacers fixed in place.
         2. Insert the gel-plates pair in the gel stand vertically with the glass surface facing you.

Make sure all edges are flush or it will leak.

Make it tight, but not too tight

* + - 1. Screw the plates with the little grey screws, not too tight but just enough to hold the gel.
      2. The gel seal [contained in a small white tube] is used on the corners [edges which face opposite the open surface]. [GELSEAL 5GR TUBE or Vaseline]
         1. Put some gel across the bottom of the glass and aluminium
      3. Parafilm is used as a suspender, and a neatly cut parafilm strip is doubly folded and kept on the surface of the gel caster unit.
      4. Put the gel stand in the gel caster and use large black screws clamps on the sides, by turning them and pushing in and up 180 degrees [don’t over rotate them].
         1. Put red clamps on the top of the gel plate and aluminium backing plate.

**Running Gel (Two Gels – 10mL total)**

Make running gel in a small beaker

|  |  |
| --- | --- |
| Distilled Water | 10uL |
| 50% (v/v) Glycerol | 4.00 mL |
| 1.50 mol/L Tris/HCl Solution, pH 8.8 | 2.50 mL |
| 2,2,2 trichloroethanol | 50uL |
| 10% (w/v) SDS Solution | 100uL |
| 30% (w/v) Acrylamide Solution | 3.33mL |
| 10% (w/v) Ammonium Persulfate Solution | 50uL |
| TEMED (tetramethylethylenediamine) | 10uL |

* 1. Add 4.8 mL of solution to each gel caster
  2. Take a transfer pipet and fill to the top of the caster with butenol (take from top of bottle labelled Water-Sat i’o butanol)
  3. Let polymerize for 20 minutes

**Stacking Gel (Two Gels – 4mL total)**

* + - 1. Make stacking gel in small beaker

|  |  |
| --- | --- |
| Distilled Water | 1.54mL |
| 50% (v/v) Glycerol | 800uL |
| 0.50 mol/L Tris/HCl Solution, pH 6.8 | 1.00mL |
| 10% (w/v) SDS Solution | 40uL |
| 30% (w/v) Acrylamide Solution | 600uL |
| 10% (w/v) Ammonium Persulfate Solution | 20uL |
| TEMED (tetramethylethylenediamine) | 4uL |

* + - 1. Fill each cassette to the top then put a 0.75mm comb into each, pushing the comb down so it is only a few milometers from the top of the running gel
      2. Let gel polymerize for 25 minutes
      3. If you are not running the gel today, wrap the entire caster apparatus in plastic wrap and place in the fridge overnight
      4. If you are running the gels today, see Western Blot SDS Page Protocol