SDS & 2 DIMENSIONAL
GELS

PROTOCOL FOR PREPARATION OF SDS GELS FOR 2D PROTEIN SEPARATIONS

(All instructions are for a Hoefer SE600 electrophoresis apparatus)

A. MATERIALS NEEDED

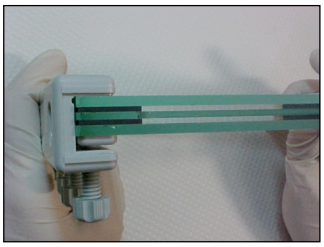
- **a.** Low Fluorescence Glass Plates.
- **b.** Bind-Silane.
- **c.** MeOH.
- **d.** Laemmli SDS PAGE buffers.
- **e.** MW standards on paper tabs.
- **f.** solutions Sealing agarose (0.5% low melting temperature agarose in 1X Laemmli stacking buffer).

B. PRETREATING LOW FLUORESCENCE GLASS PLATES WITH BIND-SILANE

1. Clean glass plates very well with MeOH.
2. Pipet 1 ml Bind-Silane onto ONE plate. DO NOT DO BOTH PLATES.
3. Rub the Bind-Silane over the entire plate surface.
4. Air dry for 1.5 hours.
5. Polish the Bind-Silane plate with a fresh Kimwipe.
6. **THIS WILL BE THE BACK PLATE OF YOUR MOLD. PAY ATTENTION.**

CASTING APPARATUS SET-UP (13X16CM GELS)

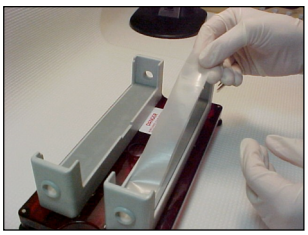
1. For each gel, select: 2 rectangular glass plates, 2-1mm (white) spacers, 2 side screw-clamps, 2 black cams, one casting stand.
2. Clean inside-facing surface of NON-BIND-SILANE TREATED glass plate with MeOH and Kimwipe.
3. Assemble gel molds; 2 rectangular plates, 2 spacers, 2 clampls. Slide on side screw-clamps.



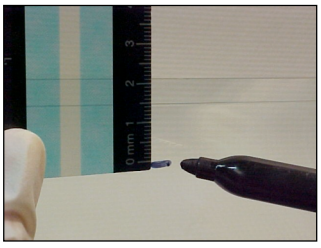
4. Align bottom edges of all components against bench, loosely tighten screw-clamps, ***make all necessary adjustments to eliminate gaps in sandwich***, tighten all screws.

5. Insert 1 gel index tab into bottom right corner of each gel. (optional)

6. Seal bottom corners and chips in glass with Vaseline as necessary.
7. Lay folded Parafilm in bottom of casting stand.

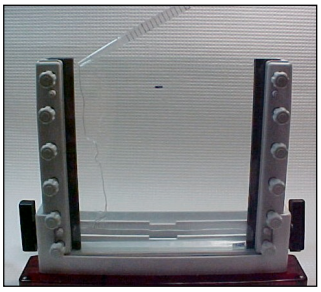


8. Slide molds into place over Parafilm layer.
9. Insert and tighten cams.
10. Mark glass 1.5cm below the top of the glass plate.

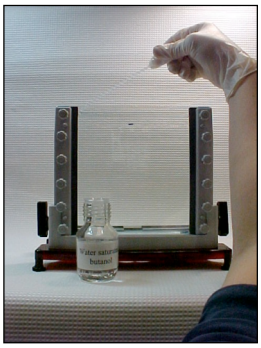


C. POUR GELS

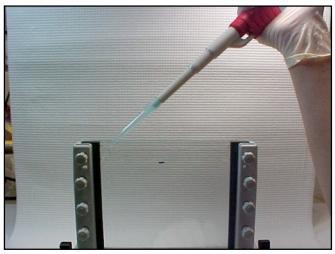
11. Make 50mL separator gel. (Enough for two gels. Concentration depends upon target MW).
12. Pipet separator solution into each side of sandwich up to mark.



13. Overlay separator solution with H_2O-saturated isobutanol, set (15-45 min).



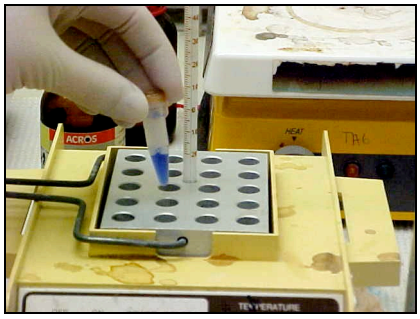
14. Pour off isobutanol, rinse surface with H_2O, dry w/ 3MM filter paper.
15. Make 5 mL stacker gel.
16. Pipet 1mL stacker onto gel.



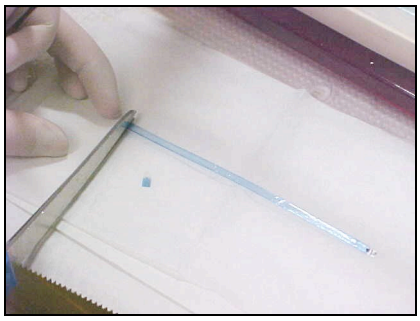
17. Overlay stacker w/ H_2O-saturated isobutanol, set (10-15 min).
18. Pour off isobutanol, rinse w/ H_2O, carefully dry **without** touching stacker surface.

D. LOAD FOCUSED DRYSTRIPS ONTO GELS

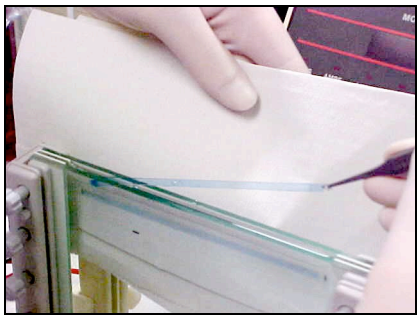
19. Turn on chiller (temp → 10C), check 10% MeOH coolant level. Melt tube of sealing agarose in 65C block. Remove Hi or Low unstained mw std tabs from –20C.



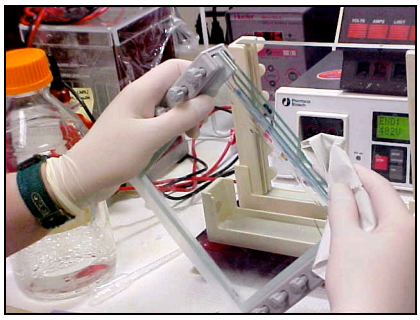
20. Fill sandwich w/ 1X SDS running buffer.
21. With forceps, lay reduced/alkylated DryStrip plastic-down on Kimwipe, cut off pointed tip at electrode mark.



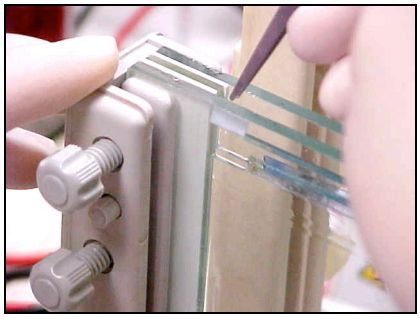
22. Position strip between glass plates, drop onto stacker surface, position ANODE end of strip on left (as you face the gel). The gel should be positioned as far to the right of the gel as possible. [repeat steps 21 and 22 for all gels]



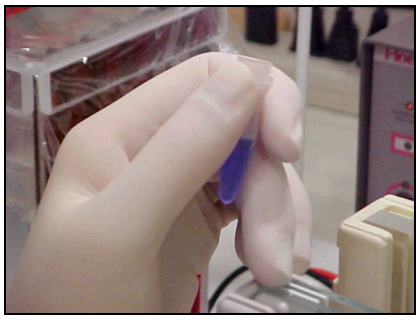
Carefully pour off buffer into paper towels. (avoid air bubbles between strip and gel surface)



23. Drop and secure 1 MW tab onto left-most stacker surface for each gel.



24. Cool sealing agarose somewhat in hand.

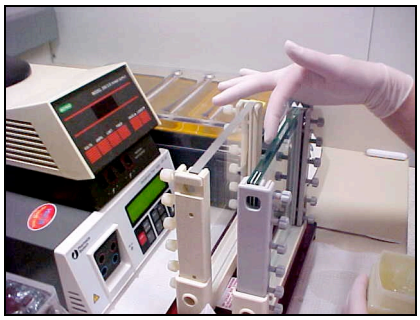


25. Drop by drop, add just enough sealing agarose to tack down IPG DryStrip and MW marker tab, allow to set momentarily; next add enough sealing agarose to cover strip.

26. Let set ~5 min.

E. RUNNING GELS

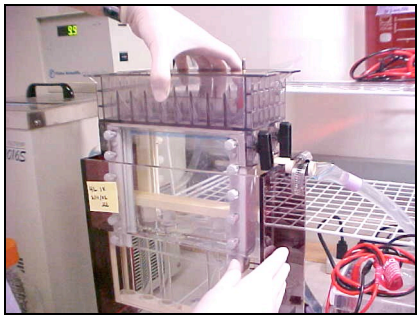
28. Seal chips in tops of glass plates with petroleum jelly.



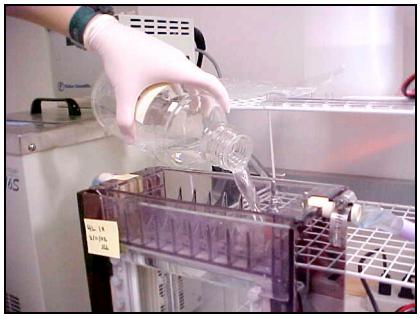
29. Attach upper buffer reservoir, tighten cams to vertical position.

30. Fill reservoir wells with 1X SDS running buffer, check for leaks around sandwich(es).

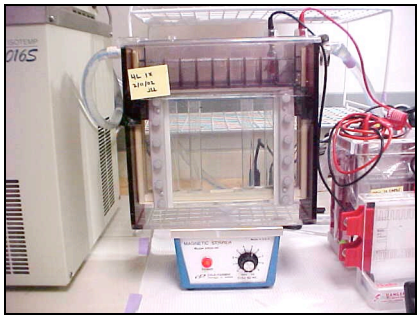
31. Lower assembly into tank of 1X SDS running buffer.



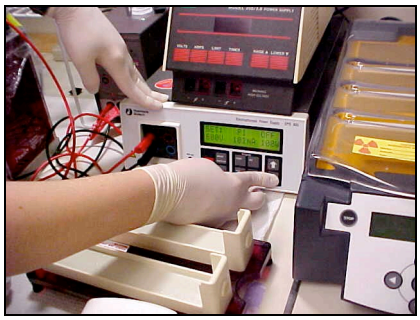
32. Carefully fill upper buffer chamber w/ 1X running (electrode), covering electrode and not disturbing loaded DryStrips.



33. Cover tank and connect electrodes to power supply.



34. Run at 35mAmps/gel (v=600, w=100).



35. Stop electrophoresis when dye front is at bottom edge of gel. [~3 hrs for large gel, ~1 hr for mini-gel]. Continue electrophoresis to run the Bromophenol Blue line off the gel (5 minutes).

36. Prepare for imaging.