

ACRYLAMIDE GEL PROTOCOL

I. Glass Preparation

1. Clean both glasses with soap and water.
2. Rinse with distilled water, dry and clean with EtOH (95%)
3. Let them dry.

a. Large glass

- i. When completely dry, treat with bind silene solution. This procedure has to be done in a Fume Hood.
- ii. Spread the bind silene solution evenly throughout the glass and let it dry in the fume hood 15-30 min.

Bind Silene solution preparation

Mix in a 15 ml plastic tube.

1. 5 ul of Bind Silene
2. 5 ul of Acetic Acid
3. 5 ml of 95% EtOH.

b. Small glass

- i. When completely dry, treat with Rain-X (Water repellent liquid).
- ii. Spread the Rain X evenly throughout the glass and dry using Kimwipes.

II. Gel preparation

1. Mix the following reagents in a beaker (use a stir plate)

Total Volume	100 mL	100 ml	80 mL	80 ml
Concentration	8 %	6.5 %	8 %	6 %
Reagents				
40% Acrylamide/bis soln	20.0ml	16.25ml	16ml	12ml
Urea (7-8 M)	45.045g	45.045g	36.036g	36.036g
TBE 10 X buffer	10.0ml	10.00ml	8.0ml	8.0ml
dH2O	up to 100ml	up to100ml	up to 80ml	± 35ml

2. Add the proper amount of Urea depending on the gel concentration.
3. Add the Acrylamide solution (Stored at 4°C and pre-diluted to 40%, Add Amberlite and filter).
4. Add an estimate amount of distilled water (35 ml if the final vol is 80 ml and the gel is 6%)

5. Leave on stirrer plate until the urea is completely dissolved.

****Setting up the plates can be done while urea is dissolving.**

PLATE SETUP

Before setting up plates, ensure no liquid or particles on the their surface. Clean with Ethanol using KimWipe.

- a. Put the spacers on the big glass (the thick of the spacers is 0.4 mm).
 - b. Put the small glass on top and then the casting boot; be sure that the casting boot is closed at the bottom. Clips can be used to press the glass together while you are putting the casting boot.
6. When the urea is completely dissolved add an estimate amount (a small scoop) of Amberlite. (You may skip this step by adding the Amberlite to the 40% Acrylamide stock solution).
 7. Stir the Amberlite for 5 minutes.
 8. Setup the Vacuum, put a new filter and close tightly with a 250ml Pyrex bottle.
 9. First, Filter the 10x TBE buffer and then the Urea/Acrylamide solution. Put the beaker inside an ice bucket while solution is filtering (to delay polymerization) and take out when ready to add Temed and APS*.
 10. Bring filtered solution up to 80ml with ddH₂O.
 11. Add 100uL of TEMED and 400uL APS, swirl beaker continuously while adding for it to mix well.
 12. Pour gel mix into squeeze bottle and pour the gel immediately.

***APS preparation (10% Ammonium Persulfate)**

0.1g of APS
ddH₂O up to 1000uL

III. Pour Gel

1. Pour gel softly and consistently, DON'T STOP.
2. Once the gel fills the space between glasses (Without bubbles) put the combs in, cover with clingfilm and clip the wrap to the glasses with 2 or 3 clips.
3. Leave to polymerize 2.5-3 hrs (18hrs max).
4. If leaving for more than 8 hours put a wet kimwipe on the border of the gel, where the comb is, to prevent over drying.