

LAEMMLI GEL STOCKS

30% Acrylamide + 0.8% Bis:

	<u>200mL</u>
Acrylamide	60.0g
Bis	1.6g

- Dissolve in 150 mL milliQ H₂O.
- Bring up to volume. Stir.
- Filter with Whatman paper.
- Store in a brown bottle (or cover bottle with foil) and keep in refrigerator.

4x Laemmli Lower Tris (1.5M Tris + 0.4% SDS):

	<u>500mL</u>
Tris Base	90.83g
10% SDS	20mL

- Dissolve in 400mL milliQ H₂O.
- Note: Tris pKa is sensitive to both temperature and concentration.
- Bring up to volume.
- Add concentrated HCl to pH to 8.8. Note: you must use a pH electrode that is sensitive to Tris. It equilibrates slowly.
- Use high quality SDS (e.g. from Biorad)

4x Laemmli Upper Tris (0.5M Tris + 0.4% SDS):

	<u>250mL</u>
Tris Base	15.14g
10% SDS	10mL

- Dissolve in 150mL milliQ H₂O.
- Bring up to volume.
- Add concentrated HCl to pH to 6.8. Note: you must use a pH electrode that is sensitive to Tris. It equilibrates slowly.
- Use high quality SDS (ex. from Biorad)

Laemmli Reservoir Buffer:

	<u>1000mL</u>
Tris Base	3g
Glycine	14.4g
10% SDS (Biorad)	10mL

Laemmli Sample Buffer:

	<u>2x (50mL)</u>	<u>4x (5mL)</u>
Glycerol	5mL	1.0mL
10% SDS	15mL	3.0mL

4x Upper Tris	6.25 mL	1.25mL
Bromphenol Blue	0.05g	0.1g
*H ₂ O	18.75mL	

- For 4x sample buffer, can use 200µL 1% Bromphenol blue.

*Note:

- For reducing sample buffer add B-ME to 10% just prior to use
- If you wish to leave out the B-ME, add $\frac{1}{10}$ volume of H₂O just prior to use
- You can also add DTT

Table for Making Laemmli Gels:

Lower Gel – 40 mL

	<u>5%</u>	<u>7.5%</u>	<u>8.75%</u>	<u>10%</u>	<u>12%</u>	<u>15%</u>
H ₂ O	22.7	18.35	17.68	16.12	12.72	9.42
Lower Tris	10.0	10.0	10.0	10.0	10.0	10.0
Laemmli Acrylamide 30%	6.7	10.0	11.7	13.3	16.6	20.0
10% APS	120µL	120µL	120µL	120µL	120µL	120µL
TEMED	20µL	15µL	10µL	10µL	10µL	10µL

- Takes about $\frac{1}{2}$ hour to polymerize. Can use more APS and TEMED to hasten polymerization.

Upper Gel – 20mL

	<u>3%</u>	<u>5%</u>
H ₂ O	12.68	11.35
Upper Tris	5.0	5.0
Laemmli Acrylamide 30%	2.0	3.33
10% APS	60µL	60µL
TEMED	20µL	20µL