

SDS-PAGE FOR PROTEINS

Recipes:

12.5% running gel/4% stacking gel]

| | <u>Large gel</u> | <u>Mini-gel</u> |
|--------------------------------|------------------|-----------------|
| A. Running gel | | |
| 30% acrylamide, 0.8% bis | 18.75ml | 4.2ml |
| dH ₂ O | 15.0ml | 3.3ml |
| 1.5M Tris, pH 8.8/ 0.4% SDS | 11.25ml | 2.5ml |
| TEMED | 7.5µl | 5.0µl |
| 10% Ammonium Persulfate | 150µl | 50µl |
| | | |
| B. Stacking Gel | | |
| 30% Acryl/ 0.8% Bis | 0.75ml | 1.3ml* |
| dH ₂ O | 4.75ml | 6.1ml |
| 0.5M Tris pH 6.8 | 1.88ml | 2.5ml |
| 10% SDS | 75µl | 100µl |
| TEMED | 3.2µl | 10µl |
| 10% Amm. Pers. | 75µl | 50µl |

*Makes enough for two gels

Add all reagents except TEMED, filter through .45µm filter, and degas for 15 minutes. Add TEMED and pour gels.

Stocks:

1. 30% Acrylamide/ 0.8% Bis- 250ml

75g high grade Acrylamide (e.g. Bio-Rad)

2g bis-acrylamide

Add 100ml dH₂O, stir to dissolve thoroughly and q.s. to 250ml. Filter through .45µm filter and store at 4°C in foil wrapped bottle.

2. 1.5M Tris, pH 8.8/ 0.4% SDS

90.75g Tris base

2.0g SDS

pH to 8.8 with HCl, q.s. to 500ml. Filter.

3. 0.5M Tris pH 6.8

30.25g Tris base

pH to 6.8 with HCl, to 500ml with H₂O

4. 10% ammonium persulfate (w/v)
Make fresh.

5. SDS Running Buffer
per liter

14.4g glycine
3.03g tris base
1.0g SDS

6. SDS Sample Buffer (2X)

| | |
|-------------|-------|
| Tris base | .379g |
| Glycerol | 5g |
| β -ME | 2.5ml |
| SDS | 1.15g |

pH to 6.8 with HCl to 50ml with H₂O
Add 0.05g bromophenol blue