

SDS-PAGE (Laemmli buffer system)

I. Solutions

(A) **30% Acrylamide Solution.**

30 g acrylamide
0.8 g N'N'-bis-methylene-acrylamide

Dilute to 100 mls with deionized water. Store in the dark at 4° C.

(B) **Lower Tris Buffer (4x)**

18.17 g Tris base
4.0 ml 10% SDS

Adjust pH to 8.8 with conc. HCl. Dilute to 100ml. Store at 4° C.

(C) **Upper Tris Buffer (4x)**

6.06 g Tris base
4.0 ml 10% SDS

Adjust pH to **6.8** with conc HCl. Dilute to 100 ml. Store at 4° C.

(D) **Tris-glycine reservoir buffer (4x)**

12 g Tris base
57.6 g glycine

Dilute to 1000 ml. Store at 4° C.

(E) **Sample buffer (5x)**

3.9 ml deionized water
1.0 ml 0.5 M Tris, pH 6.8
0.8 ml Glycerol
1.6 ml 10% SDS
0.4 ml 2-mercaptoethanol
0.4 ml 1% bromophenol blue

Store in freezer

(F) **10% SDS**

Dissolve 50 g SDS in 450 ml deionized water with gentle stirring and bring to 500 ml. *Store at room temperature.*

(G) **Ammonium persulfate solution (APS)** – *make fresh daily.*

Dissolve 0.1 g APS (electrophoresis grade) in 1 ml deionized water.

II. Gel preparation

(A) Resolving gel (lower gel).

	15%	12%	10%	7.5%
Deionized H ₂ O	2.45 ml	3.45 ml	4.15	4.95
4x Lower Tris Buffer	2.5 ml	2.5 ml	2.5 ml	2.5 ml
30% Acrylamide Solution	5.0 ml	4.0 ml	3.3 ml	2.5 ml
10% APS	50 µl	50 µl	50 µl	50 µl
TEMED	5.0 µl	5.0 µl	5.0 µl	5.0 µl

(B) Stacking gel (upper gel).

Deionized H ₂ O	3.05 ml
4x Upper Tris Buffer	1.25 ml
30% Acrylamide	0.67 ml
10% APS	25 µl
TEMED	<u>5.0 µl</u>

(C) Electrode Buffer

370 ml Deionized H₂O
125 ml 4x Reservoir buffer
5 ml 10% SDS

III. Stain/Destain

400 ml Methanol
100 ml Acetic Acid (glacial)
500 ml H₂O
1 g Coomassie blue R-250

Destain is the same, minus the Coomassie blue.