

Yu Lab Buffer Recipes

Updated on 6/20/03

SDS Sample Buffer (2X):

2.9 g SDS
0.4 g Tris•base
12 mL glycerol
40 mg bromophenol blue
620 mg DTT
ddw to 40 mL

Coomassie Blue Stain Solution: (4L)

2 g Coomassie Blue
2 L MeOH
1.6 L ddw
400 mL glacial acetic acid

SDS-PAGE Running Buffer (10X):

600 g Tris•base
1440 g Glycine
H₂O to 10 L

For 1X Running Buffer:

1 L 10X buffer
10 g SDS
H₂O to 10 L

10% SDS Resolving Gel:

125 mL 40% acrylamide
187.5 mL 1M Tris (pH 8.8)
5.0 mL 10% SDS
182.5 mL H₂O
500 mL TOTAL

For 10 mini-gels, add

40 mL 10% resolving gel
20 μ L TEMED
250 μ L 10% APS

Pour into multi-casting apparatus. Overlay with 0.2 mL butanol each gel

SDS Stacking Gel:

40 mL acrylamide/bis acrylamide
30 mL 1M Tris (6.8 pH)
1.67 mL 10% SDS
ddw to 250 mL

For gels, to 5 mL each, add

7 μ L TEMED
70 μ L 10% APS

15% SDS Resolving Gel:

187.5 mL 40% acrylamide/bis acrylamide (37.5:1)
187.5 mL 1M Tris (8.8 pH)
5.0 mL 100% SDS
120 mL H₂O
500 mL TOTAL

For gels, to 20 mL each, add

7 μ L TEMED
100 μ L 10% APS

6X DNA loading buffer:

100 mg bromophenol blue
100 mg xylene cyanol FF
6 g Ficoll
ddw to 40 mL

For analysis of small DNA fragment (≤ 100 bp), omit bromophenol blue, as it will interfere with ethidium bromide stain.

10X PBS:

1.44 g KH_2PO_4
90.00 g NaCl
7.95 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (or 4.21 g Na_2HPO_4)
Adjust pH to 7.4
ddw to 1 L

20X SSPE (per liter):

175.3 g NaCl
27.6 g $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$
7.4 g EDTA
Adjust pH to 7.4 with NaOH
ddw to 1 L

Western Transfer Buffer (Ref. p148. LKB Multiphor Book):

continuous 0.8 mA/cm² - NOT OVER 150 mA
2.93 g Glycine (39 mM)
5.81 g Tris-Base (48 mM)
0.375 g SDS
200 mL MeOH
ddw to 1 L

Make up Glycine, Tris•Base, SDS, 10X, dilute and add MeOH to make up 1X

Filter Washing Solution (2X SSPE, 0.2% SDS):

100 mL 20X SSPE
20 mL 10% SDS
ddw to 1 L
(2X SSPE, 0.2% SDS)

Urea Sample Buffer:

(120 mM Tris-Hcl, pH 6.8; 4% SDS; 4M urea; 20% glycerol)

4.8 mL 1M Tris-Hcl (pH 6.8)
16 mL 10% SDS
9.6 g urea
8 mL glycerol
40 mg bromophenol blue
ddw to 40 mL

LB Medium:

10 g Bacto-tryptone
5 g Bacto-yeast extract
10 g NaCl
ddw to 1 L

1000X Ampicilin (100 mg/mL):

1 g Ampicilin
Add H₂O to 10 mL, Filter

1000X Kanamycin (30 mg/mL):

300 mg Kanamycin
Add H₂O to 10 mL, Filter

1000X IPTG (1M):

2.382 g IPTG

Add H₂O to 10 mL, Filter**NZY Broth (per liter):**

5 g NaCl

2 g MgSO₄•H₂O

5 g yeast extract

10 g NZ amine (casein hydrolysate)

Adjust pH to 7.5 with NaOH

25X MMR (4 L):

2.5M NaCl 584.4 g

50mM KCl 14.9 g

25mM MgCl₂ 20.33 g MgSO₄•6H₂O50mM CaCl₂ 29.4 g CaCl₂•2H₂O

2.5mM EDTA pH 8 20 mL 0.5M EDTA

125mM HEPES pH 7.8 500 mL 1M HEPES

Prehybridization Solution (high stringency):

100 mL

10 mL 50X Denhardtts solution

20 mL formamide

30 mL 20X SSPE

1 mL salmon sperm DNA (10 mg/mL, Denatured before use)

ddw to 100 mL

(Final concentration: 5X Denhardtts; 20% formamide; 6X SSPE; 100 µg/mL

SS DNA)

20X TBS (25mM Tris final):

160 g NaCl
4 g KCl
60 g Tris•base
pH to 8.0 with HCl
ddw to 1L

Energy Mix (20X):

100 mL
3.827 g phosphocreatine (150mM)
1.102 g ATP (20mM)
0.4 mL 0.5M EGTA (pH 7.7) (2mM)
2 mL 1M MgCl₂ (20mM)
pH to 7.7 with 1N NaOH
Aliquot and store at -20°C

TAE (Tris-acetate) Buffer (50X):

per liter
242 g Tris base
57.1 mL glacial acetic acid
100 mL 0.5M EDTA (pH 8.0)