Buffer Formulations

Protocol

Bulletin 6199

Sample Preparation Buffers

1 M Tris-HCl, pH 7.6 (100 ml)

Tris base	12.11 g
Deionized H_2O (diH_2O)	80 ml
Adjust pH to 7.6 with HCl	
diH_2O	to 100 ml

0.5 M Tris-HCl, pH 6.8 (100 ml)
(catalog #161-0799)

Tris base	6.06 g
diH_2O	~60 ml
Adjust to pH 6.8 with HCl	
diH_2O	to 100 ml
Store at 4°C	

10% SDS (10 ml)
(catalog #161-0416)

SDS	1.00 g
diH_2O	to 10 ml

1.0% Bromophenol Blue (10 ml)
(catalog #161-0404)

Bromophenol blue	100 mg
diH_2O	to 10 ml

RIPA Solubilization Buffer (100 ml)
25 mM Tris-HCl pH 7.6, 150 mM NaCl, 5 mM EDTA, 1% NP-40 or 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS

NaCl	0.88 g
EDTA	0.15 g
NP-40 or Triton X-100	1 g
Sodium deoxycholate	1 g
SDS	0.10 g
diH_2O	80 ml
1 M Tris-HCl, pH 7.6	2.5 ml
diH_2O	to 100 ml

Phosphate Buffered Saline (PBS, 1 L)
0.9% (w/v) sodium chloride in 10 mM phosphate buffer, pH 7.4

NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g
diH_2O	800 ml
Adjust pH to 7.4 with HCl or NaOH	
diH_2O	to 1 L

SDS-PAGE Sample Buffer (2×, 8 ml)
(catalog #161-0737, 30 ml)
62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue

0.5 mM Tris-HCl, pH 6.8	1.0 ml
25% Glycerol	2.0 ml
1.0% Bromophenol blue	0.08 ml
10% SDS	1.6 ml
diH_2O	2.92 ml

Store as 1–2 ml aliquots at –70°C and add β-mercaptoethanol (0.4 ml) or 3% DTT immediately before use.

Protein Precipitation Solution (100 ml)
20% Trichloroacetic acid (TCA), 0.2% DTT in ice-cold acetone (–20°C)

TCA	20.00 g
DTT	0.20 g
Acetone	80 ml
Dissolve	
Acetone	to 100 ml

Store at –20°C

Wash Solution (100 ml)
0.2% DTT in ice-cold acetone (–20°C)

DTT	0.20 g
Acetone	80 ml
Dissolve	
Acetone	to 100 ml

Store at –20°C

Lysis Buffer (50 ml)
2 M thiourea, 7 M urea, 4% (w/v) CHAPS, 1% (w/v) DTT, 2% (v/v) carrier ampholytes (pH 3–10)

Urea	21.00 g
Thiourea	7.60 g
diH_2O	to 45 ml
CHAPS	2.00 g
Bio-Lyte® ampholytes	1.0 ml
DTT	0.50 g
diH_2O	to 50 ml

SDS Sample Solubilization Buffer (50 ml)
1% (w/v) SDS, 100 mM Tris-HCl (pH 9.5)

SDS	0.50 g
Tris base	0.60 g
diH_2O	40 ml
Titrate to pH 9.5 with diluted HCl	
diH_2O	to 50 ml

BIO-RAD

Gel Casting Reagents

Acrylamide/Bis (30% T, 2.67% C)

Acrylamide (29.2 g/100 ml)	87.60 g
N'N'-bis-methylene-acrylamide	2.40 g
diH_2O	to 300 ml

Filter and store at 4ºC in the dark (30 days).

Premade alternatives:
Catalog #161-0125, 37.5:1 acrylamide/bis powder
Catalog #161-0158, 30% acrylamide/bis solution

1.5 M Tris-HCl, pH 8.8 (150 ml)
(catalog #161-0798, 1 L)

Tris base (18.15 g/100 ml)	27.23 g
diH_2O	80 ml
Adjust to pH 8.8 with 6 N HCl	
diH_2O	to 150 ml

Store at 4ºC

0.5 M Tris-HCl, pH 6.8
(catalog #161-0799, 1 L)

Tris base	6.00 g
diH_2O	60 ml
Adjust to pH 6.87 with 6 N HCl	
diH_2O	to 100 ml

Store at 4ºC

10% (w/v) SDS (100 ml)
(catalog #161-0416)

SDS	10.00 g
diH_2O	90 ml
Dissolve with gentle stirring	
diH_2O	to 100 ml

10% (w/v) APS (fresh daily)
(catalog #161-0416)

Ammonium persulfate	0.10 g
diH_2O	1 ml

Water-Saturated *n*-Butanol

n-Butanol	50 ml
diH_2O	5 ml

Combine in a bottle and shake. Use the top phase only. Store at room temperature.

Sample Buffers

2× SDS-PAGE (Laemmli, 30 ml)
(catalog #161-0737)

62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol (added fresh)

0.5 M Tris-HCl, pH 6.8	3.75 ml
50% Glycerol	15.0 ml
1.0% Bromophenol blue	0.3 ml
10% SDS	6.0 ml
diH_2O	to 30 ml

β-mercaptoethanol (50 µl to 950 µl sample buffer) before use.

2× Native PAGE (30 ml)
(catalog #161-0738)

62.5 mM Tris-HCl, pH 6.8, 40% glycerol, 0.01% bromophenol blue

0.5 M Tris-HCl, pH 6.8	3.75 ml
50% Glycerol	24.0 ml
1.0% Bromophenol blue	0.3 ml
diH_2O	to 30 ml

2× Tricine (30 ml)
(catalog #161-0739)

200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% b-mercaptoethanol (added fresh)

0.5 M Tris-HCl, pH 6.8	12.0 ml
50% Glycerol	24.0 ml
10% SDS	6.0 ml
Coomassie Blue G-250	12.00 mg
diH_2O	to 30 ml

β-mercaptoethanol (20 µl to 980 µl sample buffer) before use.

Running Buffers

10× SDS-PAGE (1 L)
(cata log #161-0732)

250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3	
Tris base	30.30 g
Glycine	144.10 g
SDS	10.00 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3).

10× Native PAGE (1 L)
(catalog #161-0734)

250 mM Tris, 1.92 M glycine, pH 8.3	
Tris base	30.30 g
Glycine	144.10 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3).

10× Tris-Tricine (1 L)
(catalog #161-0744)

1 M Tris, 1 M Tricine, 1% SDS, pH 8.3	
Tris base	121.10 g
Tricine	179.20 g
SDS	10.00 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3).

Buffer Components

0.5 M Tris-HCl, pH 6.8 (1 L)
(catalog #161-0799)

Tris base	60.60 g
diH_2O	~900 ml
Adjust to pH 6.8 with HCl	
diH_2O	to 1 L

Store at 4°C

10% SDS (250 ml)
(catalog #161-0416)

SDS	25.00 g
diH_2O	to 250 ml

1.0% Bromophenol Blue (10 ml)
(catalog #161-0404)

Bromophenol blue	100 mg
diH_2O	to 10 ml

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6640).

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