Protein electrophoresis and western blot recipes

Stock solutions
- 1 M Tris-HCl, pH 7.6
- 0.5 M Tris-HCl, pH 6.8
- 10% SDS
- 1.0% bromophenol blue
- 10X Tris-buffered saline (TBS)
- 10X phosphate-buffered saline (PBS)

Sample preparation buffers
- RIPA buffer
- 2X SDS sample buffer (Laemmli buffer)
- 4X LDS sample buffer

Electrophoresis running buffers
- 10X Tris-glycine SDS running buffer
- 10X Tris-glycine native running buffer
- 20X MOPS SDS running buffer
- 20X MES SDS running buffer
- 10X tricine SDS running buffer

Transfer buffer
- 25X Tris-glycine transfer buffer
- 20X Bis-Tris transfer buffer

Wash buffers
- Tris-buffered saline with Tween™ 20 surfactant (TBST)
- Phosphate-buffered saline with Tween 20 surfactant (PBST)

Blocking and stripping buffers
- 5% nonfat milk
- 3% BSA
- Stripping buffer

Gel casting recipes
- Invitrogen™ SureCast™ reagents
- Stand-alone reagents

Stock solutions

1 M Tris-HCl, pH 7.6

Tris base	12.11 g
Deionized water	80 mL
Adjust to pH 7.6 with HCl	
Deionized water	to 100 mL

0.5 M Tris-HCl, pH 6.8

Tris base	6.06 g
Deionized water	60 mL
Adjust to pH 6.8 with HCl	
Deionized water	to 100 mL

10% SDS

SDS	1.00 g
Deionized water	to 10 mL

1.0% bromophenol blue

Bromophenol blue	100 mg
Deionized water	to 10 mL

10X Tris-buffered saline (TBS)

Tris base	24 g
NaCl	88 g
Deionized water	900 mL
Adjust to pH 7.6 with HCl	
Deionized water	to 1,000 mL

10X phosphate-buffered saline (PBS)

NaCl	80 g
KCl	2 g
Na_2HPO_4	14.4 g
NaH_2PO_4	2.4 g
Deionized water	900 mL
Adjust to pH 7.0 with NaOH	
Deionized water	to 1,000 mL

Ready-to-use alternatives:

Thermo Scientific™ Pierce™ 20X TBS Buffer (Cat. No. 28358)

Thermo Scientific™ Pierce™ 20X PBS Buffer (Cat. No. 28348)

Sample preparation buffers

RIPA buffer: 25 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS (100 mL), pH 7.6

NaCl	0.88 g
NP-40	1 g
Sodium deoxycholate	1 g
10% SDS	1 mL
1 M Tris-HCl, pH 7.6	2.5 mL
Deionized water	to 100 mL
Thermo Scientific™ Pierce™ Protease Inhibitor Tablet (Cat. No. A32965)	2 tablets

Ready-to-use alternative:

Thermo Scientific™ RIPA Lysis and Extraction Buffer (Cat. No. 89900)

SDS sample buffer (Laemmli buffer): 63 mM Tris-HCl, 10% glycerol, 2% SDS, 0.0025% bromophenol blue, pH 6.8

Recipe for 2X buffer stock:

0.5 M Tris-HCl, pH 6.8	2.5 mL
Glycerol	2 mL
10% (w/v) SDS	4 mL
0.1% (w/v) bromophenol blue	0.5 mL
Deionized water	to 10 mL

The buffer is stable for 6 months when stored at 4°C.

Ready-to-use alternative:

Invitrogen™ Novex™ Tris-Glycine SDS Sample Buffer (2X) (Cat. No. LC2676)

Sample preparation buffers

LDS sample buffer: 106 mM Tris-HCl, 141 mM Tris base, 2% LDS, 10% glycerol, 0.51 mM EDTA, 0.22 mM SERVA Blue G250, 0.175 mM phenol red, pH 8.5
Recipe for 4X buffer stock:

Tris HCl	0.666 g
Tris base	0.682 g
LDS	0.800 g
EDTA	0.006 g
Glycerol	4 g
SERVA Blue G250 (1% solution)	0.75 mL
Phenol red (1% solution)	0.25 mL
Deionized water	to 10 mL

The buffer is stable for 6 months when stored at 4°C. Do not use acid or base to adjust pH.

Ready-to-use alternative:

Invitrogen™ NuPAGE™ LDS Sample Buffer (4X) (Cat. No. NP0007)

Electrophoresis running buffers

Tris-glycine SDS running buffer: 25 mM Tris base, 192 mM glycine, 0.1% SDS, pH 8.3
Recipe for 10X buffer stock:

Tris base	29 g
Glycine	144 g
SDS	10 g
Deionized water	to 1,000 mL

Ready-to-use alternative:

Invitrogen™ Novex™ Tris-Glycine SDS Running Buffer (10X) (Cat. No. LC2675)

Tris-glycine native running buffer: 25 mM Tris base, 192 mM glycine, pH 8.3
Recipe for 10X buffer stock:

Tris base	29 g
Glycine	144 g
Deionized water	to 1,000 mL

Ready-to-use alternative:

Invitrogen™ Novex™ Tris-Glycine Native Running Buffer (10X) (Cat. No. LC2672)

MOPS SDS running buffer: 50 mM MOPS, 50 mM Tris base, 0.1% SDS, 1 mM EDTA, pH 7.7
Recipe for 20X buffer stock:

MOPS	104.6 g
Tris base	60.6 g
SDS	10 g
EDTA	3.0 g
Deionized water	to 500 mL

Ready-to-use alternative:

Invitrogen™ NuPAGE™ MOPS SDS Running Buffer (20X) (Cat. No. NP0001)

Electrophoresis running buffers

MES SDS running buffer: 50 mM MES, 50 mM Tris base, 0.1% SDS, 1 mM EDTA, pH 7.3
Recipe for 20X buffer stock:

MES	97.6 g
Tris base	60.6 g
SDS	10 g
EDTA	3.0 g
Deionized water	to 500 mL

Do not use acid or base to adjust pH.

Ready-to-use alternative:

Invitrogen™ NuPAGE™ MES SDS Running Buffer (20X) (Cat. No. NP0002)

Tricine SDS running buffer: 100 mM Tris base, 100 mM tricine, 0.1% SDS, pH 8.3
Recipe for 10X buffer stock:

Tris base	121 g
Tricine	179 g
SDS	10 g
Deionized water	to 1,000 mL

The buffer is stable for 6 months when stored at room temperature. Do not use acid or base to adjust pH.

Ready-to-use alternative:

Invitrogen™ Novex™ Tricine SDS Running Buffer (10X) (Cat. No. LC1675)

Transfer buffers

Tris-glycine transfer buffer: 12 mM Tris base, 96 mM glycine, pH 8.3
Recipe for 25X buffer stock:

Tris base	18.2 g
Glycine	90 g
Deionized water	to 500 mL

Ready-to-use alternative:

Invitrogen™ Novex™ Tris-Glycine Transfer Buffer (25X) (Cat. No. LC3675)

Bis-Tris transfer buffer: 25 mM bicine, 25 mM Bis-Tris (free base), 1 mM EDTA, pH 7.2
Recipe for 20X buffer stock:

Bicine	10.2 g
Bis-Tris (free base)	13.1 g
EDTA	0.75 g
Deionized water	to 125 mL

The buffer is stable for 6 months when stored at 4°C. Do not use acid or base to adjust pH.

Ready-to-use alternative:

Invitrogen™ NuPAGE™ Transfer Buffer (20X) (Cat. No. NP0006)

Wash buffers

Tris-buffered saline with Tween 20 surfactant (TBST)

10X TBS	100 mL
Tween 20 surfactant	1 mL
Deionized water	to 1,000 mL

Ready-to-use alternative:

Thermo Scientific™ Pierce™ 20X TBS Tween™ 20 Buffer (Cat. No. 28360)

Phosphate-buffered saline with Tween 20 surfactant (PBST)

10X PBS	100 mL
Tween 20 surfactant	1 mL
Deionized water	to 1,000 mL

Ready-to-use alternative:

Thermo Scientific™ Pierce™ 20X PBS Tween™ 20 Buffer (Cat. No. 28352)

Blocking and stripping buffers

5% nonfat milk

Nonfat dry milk	2.5 g
TBST or PBST	to 50 mL
Filter to remove particulates	

Ready-to-use alternative:

Thermo Scientific™ Pierce™ Clear Milk Blocking Buffer 10X (Cat. No. 37587)

3% BSA

BSA	1.5 g
TBST or PBST	to 50 mL
Filter to remove particulates	

Ready-to-use alternative:

Thermo Scientific™ Pierce™ Blocker BSA (10X) in TBS (Cat. No. 37520)

Thermo Scientific™ Pierce™ Blocker BSA (10X) in PBS (Cat. No. 37525)

Stripping buffer

0.5 M Tris-HCl, pH 6.8	12.5 mL
10% SDS	20 mL
2-mercaptoethanol	0.8 mL
Deionized water	67.5 mL

Ready-to-use alternatives:

Thermo Scientific™ Restore™ Western Blot Stripping Buffer (Cat. No. 21059)

Thermo Scientific™ Restore™ Fluorescent Western Blot Stripping Buffer (Cat. No. 62300)

Gel casting recipes

Recipes for SureCast reagents

The volumes provided in the table are for a single gel. Scale volumes proportionally based on the number of gels to be cast.

	Polyacrylamide concentration								
Separating gel solution	**4%**	**6%**	**8%**	**10%**	**12%**	**14%**	**16%**	**18%**	**20%**
SureCast Acrylamide (40%) (Cat. No. HC2040)	0.8 mL	1.2 mL	1.6 mL	2.0 mL	2.4 mL	2.8 mL	3.2 mL	3.6 mL	4.0 mL
SureCast Resolving Buffer (Cat. No. HC2215)	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL
Distilled water	5.1 mL	4.7 mL	4.3 mL	3.9 mL	3.5 mL	3.1 mL	2.7 mL	2.3 mL	1.9 mL
10% SureCast APS (Cat. No. HC2005)	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL
SureCast TEMED* (Cat. No. HC2006)	8 µL	8 µL	8 µL	8 µL	8 µL	8 µL	8 µL	8 µL	8 µL

* Add this last and mix well just before the gel is to be poured.

Prepare the stacking gel solution according to the following table. The volumes provided in the table are for a single gel. Scale volumes proportionally based on the number of gels to be cast. Note: Solutions do not require degassing.

Stacking gel solution	**4%**
SureCast Acrylamide (40%) (Cat. No. HC2040)	0.30 mL
SureCast Stacking Buffer (Cat. No. HC2112)	0.75 mL
Distilled water	1.92 mL
10% SureCast APS (Cat. No. HC2005)	30 µL
SureCast TEMED* (Cat. No. HC2006)	3 µL

* Add this last and mix well just before the gel is to be poured.

Recipes for stand-alone reagents

Stock solutions

Prepare the following stock solutions. All solutions can be stored at room temperature.

50% acrylamide/Bis (29:1) • 48.3 g acrylamide • 1.7 g Bis Bring to 100 mL with water Store up to two months in a dark glass bottle	**Separating gel buffer (1 M Tris-HCl, pH 8.8)** • Add 30.3 g Tris to 150 mL water • Adjust to pH 8.8 with HCl Bring to 250 mL with water	**Stacking gel buffer (0.375 M Tris-HCl, pH 6.8)** • Add 11.4 g Tris to 150 mL water • Adjust to pH 6.8 with HCl Bring to 250 mL with water	**Catalyst: ammonium persulfate (APS) (make fresh the day of use)** • 100 mg ammonium persulfate Bring to 2 mL with water
10% SDS • 10.0 g SDS Bring to 100 mL with water	**50% sucrose** • 50.0 g sucrose Bring to 100 mL with water		

Separating gel

The volumes provided in each column are for approximately 25 mL of separating gel, enough for four 1.0 mm thick mini gels. Scale volumes proportionally based on the number of gels to be cast.

Solution	6% gel	8% gel	10% gel	12% gel	14% gel	16% gel	18% gel	20% gel
50% acrylamide/Bis	3.0 mL	4.0 mL	5.0 mL	6.0 mL	7.0 mL	8.0 mL	9.0 mL	10.0 mL
Separating gel buffer	9.4 mL	9.4 mL	9.4 mL	9.4 mL	9.4 mL	9.4 mL	9.4 mL	9.4 mL
10% SDS	250 µL	250 µL	250 µL	250 µL	250 µL	250 µL	250 µL	250 µL
50% sucrose*	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Water	7.8 mL	6.8 mL	5.8 mL	4.8 mL	3.7 mL	2.7 mL	1.7 mL	750 µL
TEMED**	6.25 µL	6.25 µL	6.25 µL	6.25 µL	6.25 µL	6.25 µL	6.25 µL	6.25 µL
APS**	625 µL	625 µL	625 µL	625 µL	625 µL	625 µL	625 µL	625 µL

* Optional but recommended because it makes it easy to form a good interface between the separating gel and the overlay. If omitted, increase the amount of water added to make up for the volume of the sucrose solution (increase the water by 4.0 mL for the above tables).

** Add these last and mix well just before the gel is to be poured.

Stacking gel

The following recipe is for a 4% stacking gel (12.5 mL).

Solution	4%
50% acrylamide/Bis	1.0 mL
Stacking gel buffer	4.2 mL
10% SDS	125 µL
Water	6.3 mL
TEMED**	5.0 µL
APS**	1.0 mL

** Add these last and mix well just before the gel is to be poured.

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