Buffer Preparation (Shi Lab)

1. 1 M Tris-HCl Buffers

pH	Volume (L)	TrisBase (g)	HCl (ml)
pH 7.0	2	242.2	150-155
pH 7.5	2	242.2	120-125
pH 8.0	2	242.2	80-85

Autoclavable.

2. EDTA 0.5 M (pH8.0)

0.5M, 1L: 148 g EDTA
+ ~30-40 g NaOH to adjust pH
(or 186 g EDTA-Na.2H2O + ~20 g NaOH)
Note: pH adjusted by NaOH is essential for solubility. Autoclavable.

3. TAE DNA Electrophoresis Buffer (50 X)

(2 M Tris, 50 mM EDTA)

4 L
968 g Tris
228.4 ml glacial acetic acid
400 ml 0.5 M EDTA 8.0

To make 1x TAE 20 L, add 400 ml 50X buffer into 19.6 L ddH2O.

4. SDS-PAGE Gel Solutions

	Vol (L)	Tris (g)	HCl (ml)	10% SDS (ml)
4x Lower gel buffer *1.5 M Tris-Cl, pH 8.8, 0.4% SDS*	2	363.3	50-60	80 ml
4x Upper gel buffer *0.5 M Tris-Cl, pH 6.8, 0.4% SDS*	2	121.1	70-80	80 ml

4.1 10% SDS

2L:
200g SDS into 2 L, heat to 68^{o}C for solubility. pH ~6.6.

5. 5X SDS Loading Sample Buffer

100 ml

	Stock solution	Add volume
250 mM TrisHCl pH6.8	1 M	25 ml
10% SDS		10 g
30% Glycerol		30 ml
5% β-mercapitalethanol (or 0.5M DTT)		*5 ml*
0.02% bromophenol blue	1%	2 ml

6. 6X DNA loading sample buffer:
(40% sucrose, 0.01-0.02% BPB)

100 ml
Add 40 g sucrose to 50 ml ddH2O, add 2 ml 1% BPB solution, adjust to 100 ml.

7. SDS-PAGE Electrophoresis Running Buffer (10x)
*(**1x**: 25 mM Tris, 192 mM glycine, 0.1% SDS, pH8.3)*

10 L.
303 g Trisbase (FW 121.1)
1440 g glycine (FW 75.07)
100 g SDS
No need to adjust pH

8. Transfer Buffer without SDS (10x)
*(**1x**: 25 mM Tris, 192 mM glycine, pH8.3)*

10 L
303 g Trisbase,
1440 g glycine
No need to adjust pH

8.1 Transfer Buffer (1x)

500 ml
50 ml of 10x SDS-PAGE running buffer
100 ml of Methanol (final **20% methanol**)
350 ml ddH2O

9. TBS (10x)

*(**1x**: 150 mM NaCl, 10 mM Tris pH8.0)*

10 L
876.6 g NaCl (FW 58.44),
121.1 g Tris,
~50-60 ml HCl
to pH8.0

9.1 TBS-T (1x)

20L
2L 10x TBS
200 ml 10% Tween20 (final 0.1% v/v)
ddH2O to 20 L

9.2 Block buffer

(5% Nonfat milk in TBS-T)
5g milk in 100 ml TBST

10. NaCl 4 M

2 L: 467.5 g NaCl. Autoclavable.

11. NaOH 10 M

0.5 L: 200 g

12. NaAc 3 M

500 ml: add 204 g NaAc.3H2O (FW 136), adjust pH by glacial acetic acid (~60 ml) to pH5.2. Autoclavable.

13. MgCl2 1M

500 ml: Add 101.65 g MgCl2.6H2O into 500 ml ddH2O. Autoclavable.

14. CaCl2 1M

400 ml: Add 58.8 g CaCl2.2H2O (FW 147), filter for sterilization.
Dilute 10x to make 100 mM CaCl2.

15. MgSO4 1M

500 ml: Add 123.3 g MgSO4.7H2O into 500 ml ddH2O. Autoclavable.

16. ZnCl 0.1M

250 ml: 3.4 g ZnCl.

Stock in -20°C

1. IPTG (1 M)

1 g IPTG (FW 238.3) resolved in 4.2 ml (~4 ml) ddH2O, filter through 0.22 μm filters, aliquot 1 ml in eppendorf. Store at -20°C.

2. DTT (1 M)

5 g DTT (FW 154.25) resolved in 32.5 ml (~30 ml)10 mM NaAc (pH 5.2), filter through 0.22 μm filters, aliquot 1 ml in eppendorf. Store at -20°C.

3. X-gal (20mg/ml)

Add 5 ml (~4.8 ml) **DMSO** into 100 mg X-gal bottom (FW 408.24). Store at -20°C.

4. PMSF (100 mM, =17.4 mg/ml)

Resolve 1.74g PMSF (MW 174) in **isoproponal**, total 100 ml. Aliquot and store at -20°C or R.T..

5. Carbencillin or Ampcillin (50 mg/ml) in water. 1000x

2.5 g 50 ml.

6. Kanamycin (10 mg/ml) in water. 200x

0.5 g 50 ml.

7. Chloramphenicol (34 mg/ml) in ethanol. 200x

1.7 g/ 50 m l.

8. lysozyme 50 mg/ml, 1000x.

2.5 g/ 50 ml.

9. TSA (MW 303):

Add 1.32 ml Ethanol into each vial (1 mg?) to make the TSA stock 2.5 mM, 5000x. Final concentration of TSA in the cell culture is 0.5 μM (~150 ng/ml).

Solutions.

1. Bacteria lysis buffer (GST pull-dwon binding buffer)
(50 mM Tris 7.5, 150 mM NaCl, 0.05% NP-40.)
1L
50 ml 1M Tris HCl 7.5;
37.5 ml 4 M NaCl;
5 ml 10% NP-40.
ddH2O to 1L.

1.1. GST pull-dwon binding buffer (1 M)
(50 mM Tris 7.5, 300 mM NaCl, 0.05% NP-40.)
1L
50 ml 1M Tris HCl 7.5;
75 ml 4 M NaCl;
5 ml 10% NP-40.
ddH2O to 1L.

1.2. GST pull-dwon binding buffer (1 M)
(50 mM Tris 7.5, 1 M NaCl, 1% NP-40.)
500 ml
25 ml 1M Tris HCl 7.5;
125 ml 4 M NaCl;
50 ml 10% NP-40.
ddH2O to 500ml.

2. RIPA Buffer
(50 mM TrisHCl pH7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS)
1L
50 ml 1 M Tris 7.4,
37.5 ml 4 M NaCl,
4 ml 0.5 M EDTA,
10 ml NP-40.
10 ml 10% SDS.

3. Cell Lysis Buffer (Flag-IP buffer)
(50 mM TrisHCl pH7.4, 250 mM NaCl, 0.5% Triton X100, 10% glycerol, 1 mM DTT, PMSF, PI (Roche))
1L
50 ml 1 M Tris 7.4,
62.5 ml 4 M NaCl,
5 ml Triton X-100,
1 ml 1 M DTT,
100 ml glycerol.

ChIP Solutions

ChIP Sweeling buffer	**Stock**	**Vol for 500 ml**
5 mM PIPES pH 8.0	0.5 M	5 ml
85 mM KCl	3 M	14 ml
1% NP40	10%	50 ml
ddH2O		433 ml
ChIP Nuclei Lysis buffer		**500 ml**
50 mM Tris-Cl pH 8.0	1 M	25 ml
10 mM EDTA	0.5 M	10 ml
1% SDS	10%	50 ml
ddH2O		425 ml
ChIP Dilution buffer		**500 ml**
16.7 mM Tris-Cl pH 8.0	1 M	8.4 ml
167 mM NaCl	4 M	20.8 ml
0.01% SDS	10%	0.5 ml
1.1% Trition X 100	10%	55 ml
1.2 mM EDTA	0.5 M	1.2 ml
ddH2O		414 ml
ChIP Dialysis buffer -Rabbit		**1000 ml**
50 mM Tris-Cl pH 8.0	1 M	50 ml
0.2% Sarkosyl	20%	10 ml
2 mM EDTA	0.5 M	4 ml
ddH2O		926 ml
ChIP Dialysis buffer -Mouse		**1000 ml**
50 mM Tris-Cl pH 8.0	1 M	50 ml
2 mM EDTA	0.5 M	4 ml
ddH2O		946 ml
ChIP Wash buffer-Rabbit		**1000 ml**
100 mM Tris, pH 9.0	1 M	100 ml
500 mM LiCl (MW 42.4)		21.2 g
1% NP40	10%	100 ml
1% Deoxycholic acid (sodium salt. MW 414.5)		10 g
ChIP Wash buffer-mouse		**1000 ml**
100 mM Tris, pH 8.0,	1 M	100 ml
500 mM LiCl (MW 42.4)		21.2 g
1% NP40	10%	100 ml
1% Deoxycholic acid (sodium salt. MW 414.5)		10 g
ChIP Elution buffer		**Make fresh**
50 mM NaHCO3	1 M	
1% SDS	10%	

1.25 M Glycine 200ml (MW=75) 18.8 g

0.5M PIPES 200 ml
30.2g PIPES, 17-19 ml 10 M NaOH

1 M NaHCO3 (MW 84) 4.2g/50 ml

5 ml elution buffer:
0.5 ml 10% SDS
21 mg NaHCO3