BUFFERS and MEDIAS

Coomassie Blue Staining Solution

2 g Coomassie Blue

2 L Methanol or Ethanol *

1.6 L dH_2O

400 mL Glacial acetic acid

**If you will be microwaving the gel in staining solution for rapid staining use ethanol. Use methanol for staining at room temperature. Methanol stains quicker, but is unhealthy to breath in fumes during microwaving.*

Coomassie blue Destaining Solution

100 mL Methanol or Ethanol *

850 mL dH_2O

50 mL Glacial acetic acid

**If you will be microwaving the gel in destaining solution for rapid destaining use ethanol. Use methanol for destaining at room temperature. Methanol destains quicker, but is unhealthy to breath in fumes during microwaving.*

DMEM Normal Cell Culture Media

DMEM	500 mL
FBS	55 mL (10 % final)
Antibiotic-Antimycotic	5.5 mL (1 X final)
L-glutamine	5.5 mL (1 X final)

Store at +4 ˚C

DNA Loading Buffer/Dye (6 X)

100 mg Bromophenol Blue

100 mg Xylene Cyanol FF

6 g Ficoll

dH_2O to 40 mL

Energy Mix (20 X)

3.827 g Phosphocreatine (150 mM final)

1.102 g ATP (20 mM final)

0.4 mL 0.5 M EGTA pH 7.7 (2 mM final)

2 mL 1 M $MgCl_2$ (20 mM final)

pH to 7.7 with 1N NaOH

Aliquot and store at -20 ˚C

FACS Buffer

1X PBS

2% FBS

1 mM EDTA

0.1 % Sodium Azide

Sterilize through 0.22 um filter. Store at +4 ˚C.

GST Protein Purification Lysis and Wash Buffer

50 mM Tris pH 7.7

150 mM KCl

0.1 % Triton X-100

Store at +4 ˚C

Add fresh at time of use 1 mM DTT

GST Protein Purification Elution Buffer

50 mM Tris pH 7.7

100 mM KCl

Store at +4 ˚C

Add fresh at time of use 1 mM DTT

Add fresh at time of use 10 mM Glutathione

pH back to 7.7 after glutathione addition

HCEC Cell Culture Media

		[Final]	**[Stock]**
DMEM	363 mL	3 parts	-
M199	121 mL	1 part	-
Antibiotic	5 mL	1X	100X
FBS	10 mL	2%	100%
Insulin	0.5 mL	10 ug/mL	10 mg/mL (1,000X)
Transferrin	0.5 mL	2 ug/mL	2 mg/mL (1,000X)
EGF	0.1 mL	20 ng/mL	100 ug/mL (5,000X)
Sodium Selenite	0.05 mL	5 nM	50 uM (10,000X)
Hydrocortisone	0.1 mL	1 ug/mL	5 mg/mL (5,000X)
	~500 mL		

Filter sterilize with 0.22 um membrane. Store at +4 ˚C

- To make hydrocortisone use water soluble version not Sigma H0888.
- Insulin needs to be made up in acidic water (pH 2.5).
- Everything else made in water, filtered 0.22 uM, and stored at -20 ˚C.

His-Tag Protein Purification Lysis Buffer

6.9 g $NaH_2PO_4 \cdot H_2O$ (50 mM final)

17.54 g NaCl (300 mM final)

0.68 g Imidazole (10 mM final)

Adjust pH to 8.0 with NaOH

dH_2O to 1 L

Store at +4 ˚C

His-Tag Protein Purification Wash Buffer

6.9 g $NaH_2PO_4 \cdot H_2O$ (50 mM final)

17.54 g NaCl (300 mM final)

1.36 g Imidazole (20 mM final)

Adjust pH to 8.0 with NaOH

dH_2O to 1 L

Store at +4 ˚C

His-Tag Protein Purification Elution Buffer

6.9 g $NaH_2PO_4 \cdot H_2O$ (50 mM final)

17.54 g NaCl (300 mM final)

17 g Imidazole (250 mM final)

Adjust pH to 8.0 with NaOH

dH_2O to 1 L

Store at +4 ˚C

NP-40 Cell Lysis Buffer

50 mM Tris pH 7.7

150 mM NaCl

0.5 % NP-40

Store at +4 ˚C

Add fresh at time of use 1 mM DTT

Add fresh at time of use 1X PIC (protease inhibitor cocktail)

Phosphate Buffered Saline (PBS) (10 X)

1.44 g KH_2PO_4

90 g NaCl

7.95 g Na_2HPO_4 $7H_2O$ (or 4.21 g Na_2HPO_4)

dH_2O to 1 L

Do not adjust pH. 10X should be approx. pH 6.5. Diluting to 1X pH will be 7.4.

Protein Storage Buffer

50 mM Tris pH 7.7

100 mM KCl

10 % Glycerol

Store at +4 ˚C

Add fresh at time of use 1 mM DTT

RIPA Cell Lysis Buffer

50 mM	Tris pH 7.8
150 mM	NaCl
1 %	NP-40
0.5 %	Sodium deoxycholate
0.1 %	SDS

Store at +4 ˚C

Add fresh at time of use 1 mM DTT

Add fresh at time of use 1X PIC (protease inhibitor cocktail)

SDS Sample Buffer (4 X)

5.8 g	SDS
0.8 g	Tris base
24 mL	Glycerol
80 mg	Bromophenol blue
1.24 g	DTT
dH_2O	to 40 mL total volume

Start with 13 mL dH_2O. Add SDS, Tris, Bromophenol blue, and DTT. Heat at 90 ˚C until in solution. Add 24 mL glycerol. Confirm approx. 40 mL. Make 1 mL aliquots and store at -20 ˚C.

SDS-PAGE Running Buffer

10 X Base (without SDS*):

120 g	Tris base
288 g	Glycine
dH_2O	to 2 L

For 1X Running buffer:

0.4 L 10 X Base Buffer

40 mL 10% SDS (or 4 g)

dH_2O to 4 L

**10X SDS-PAGE Running buffer can't be stored long-term in SDS. Therefore we make 10X solution containing Tris and Glycine then dilute this 10X solution in SDS and dH_2O to make final 1X running buffer.*

SSC (20 X)

175.3 g NaCl (3 M final)

88.2 g Sodium citrate (0.3 M final)

800 mL dH_2O

Adjust pH to 7.0 with few drops of 14 N HCl.

Then adjust volume to 1 L with dH_2O.

Aliquot and sterilize by autoclaving.

TAE Buffer (50 X)

242 g Tris base

57.1 mL Glacial acetic acid

100 mL 0.5 M EDTA pH 8.0

dH_2O to 1 L

TBS (20 X)

160 g NaCl

4 g KCl

60g Tris base

pH to 8.0 with HCl

dH_2O to 1 L

TBS-T (1 X)

200 mL 20 X TBS

dH_2O to 4 L

2 mL Tween-20

TE Buffer

10 mM Tris pH 7.4

1 mM EDTA

Western Transfer Buffer

10X Base:

29.3 g Glycine (39 mM final)

58.1 g Tris-base (48 mM final)

3.75 g SDS

dH_2O to 1 L

For 1 X Transfer buffer*:

100 mL 10 X Base Transfer Buffer

700 mL dH_2O

200 mL Methanol

Store at +4 ˚C

**Add the solutions in the order shown above. Do not add the methanol directly to the 10X Base Transfer Buffer.*

XB Buffer

10 mM HEPES pH 7.7

1 mM $MgCl_2$

0.1 mM $CaCl_2$

100 mM KCl

50 mM Sucrose

Store at -80 ˚C

REAGENTS

Ampicillin (100 mg/mL; 1000 X)

1 g Ampicillin (powder at -20 ˚C)

dH_2O to 10 mL

Sterilize through 0.22 um filter, aliquot, and store at -20 ˚C.

Bluo-Gal

20 mg/mL stock in DMSO is 200 X (100 ug/mL final)

BrdU

2 mM stock in dH_2O is 200 X (10 uM final)

Camptothecin

5 uM stock in DMSO is 1000 X (5 nM final)

Colcemid

2 mg/mL stock in Ethanol is 13,333X for final 150 ng/mL

DAPI

5 mg/mL stock in dH_2O is 5000 X (1ug/mL final). Stain cells for 1-2 min in DAPI-PBS. Wash once in PBS.

DTT (Dithiothreitol)

1 M stock in dH_2O is 1000 X (1 mM final)

Hygromycin B

50 mg/mL stock is 125 X (0.4 mg/mL final)

IPTG (1 M; 1000 X)

2.382 g IPTG (powder at +4 ˚C)

dH_2O to 10 mL

Sterilize through 0.22 um filter, aliquot, and store at -20 ˚C.

Kanamycin (30 mg/mL; 1000 X)

300 mg Kanamycin (powder at room temp.)

dH_2O to 10 mL

Sterilize through 0.22 um filter, aliquot, and store at -20 ˚C.

NEM (N-Ethylmaleimide)

800 mM stock in ethanol is 20 X (40 mM final). 100 mg in 1 mL ethanol gives 20 X solution.

Make solution fresh at each time of use.

Neomycin (G418)

100 mg/mL stock in dH_2O. Sterilize through 0.22 um filter and store aliquots at -20 ˚C. Most cells sensitive to 0.5 - 1 mg/mL (100-200 X), but optimal concentrations should be determine by performing a kill curve on your specific cell line.

Nocadazole

10 mg/mL stock. Dilute 1000X into media then 100X into media for final 0.1 ug/mL (100,000 dilution of stock in the end for 100 ng/mL final)

PMSF

100 mM stock in Ethanol is 100 X (1 mM final). Stock good for month in -20 ˚C, longer in -80 ˚C.

Protease Inhibitor Cocktail (PIC)

Resuspend one Sigma complete protease inhibitor cocktail tablet (S-8820) in 1 mL dH_2O by vigorous vortexing. This yields a 100 X solution. Aliquot and store at -20 ˚C. Only add PIC to buffers at time of use.

Puromycin

1 mg/mL stock in dH_2O. Sterilize through 0.22 um filter and store aliquots at -20 ˚C. Most cells sensitive to 1 ug/mL (1000 X), but optimal concentrations should be determine by performing a kill curve on your specific cell line.

Tetracycline (or Doxycycline)

1 mg/mL stock in 50% ethanol is 1000 X (1 ug/mL final)

Thymidine

200 mM stock in dH_2O is 100 X (2 mM final)