Kirschner Lab Buffers

10x PBS 100 mL pH 9.2

8 g NaCl
0.2 g KCl
1.15 g Na2HPO4
0.2 g KH2PO4

pH to 9.2 with NaOH

50 mM Sodium Bicarbonate

0.042 g in 10 mL DI H2O

pH to 9.5 with NaOH

Rhodamine-B (5 mg / mL 5x pH 9.7-10)

0.025 g in 5 mL 50 mM Sodium Bicarbonate (pH 9.7 – 10)

Store at 4C wrapped in foil

50 mM Sodium Bicarb Buffered Saline (BBS) 1 L

Stock A: 0.1 M NaHCO3 (fw 84.01)

8.4 g → 1 L
or
150 mL of 750 mM NaHCO3 → 1125 mL (pH 8.4)

Stock B: 0.1 M Na2CO3 (fw 106)

10.6 g → 1 L

BBS pH 8.5 (1 L)

500 mL Stock A
~2 mL Stock B (pH to 8.5)
8.5 g NaCl
DI H2O to 1 L

BBS pH 9.3 (1 L)

400 mL Stock A
~100 mL Stock B (pH to 9.3)
8.5 g NaCl
DI H2O to 1 L

2 M NH4Cl (10 mL)

1.06 g to 10 mL DI H2O
Filter

Prehybridization mix (40 mL)

1 % BSA	10 mL	4 % BSA
1 % SDS	2 mL	20 % SDS
0.5 M NaPO4 (pH 7.9)	20 mL	1 M NaPO4 (ph 7.9)
1 mM EDTA	400 uL	100 mM EDTA (pH 8)
100 ug / mL carrier DNA	400 uL	10 mg / mL salmon sperm DNA
	7.2 mL	DI H2O

Store frozen (-20C)

Hybridization mix

1 vol	kinase reaction
100 vol	prehybridization mix

Acrylamide / Bis (30 % / 0.8 %) (200 mL)

60 g electrophoresis grade acrylamide
1.65 g bis (electrophoresis grade)
DI H2O to 200 mL

Stir
Store at 4C wrapped in foil

Use with 10 % (wt/vol) persulfate made fresh
0.1 g persulfate in 1 mL DI H2O

Laemmli Sample Buffer (10 mL 5X)

2.5 mL	1.25 M Tris (pH 6.8)
5 mL	100 % glycerol
1 g	SDS (electrophoresis grade)
0.8 g	DTT (electrophoresis grade)
1 mL	Bromophenol Blue (1 % solution)

DI H2O to 10 mL (5X)

Laemmli Gel Buffers

Buffer I (8X 3 M pH 8.8 200 mL)

18 g Tris.HCl
58.8 g Tris Base
DI H2O to 200 mL

pH to 8.8

Buffer II (8X 1M pH 6.8 100 mL)

14.4 g Tris.HCl
1.3 g Tris Base
DI H2O to 100 mL
pH to 6.8

Laemmli Running Buffer (5X 1 L)

15.14 g Tris (mw 121.1)
72.1 g Glycine (mw 75.1)
5 g Lauryl Sulfate
DI H2O to 1 L

Store at 4C

HO Buffer without BME

250 mM NaCl
10 mM Tris (pH 7.4)

Fluorescein Working Stock (10 mg / mL 10X pH 9.7 - 10)

10 mg / mL fluorescein in
50 mM Sodium bicarbonate pH 9.7 - 10

Alcoholic KOH for plate washing

In 500 mL beaker, cover bottom with KOH pellets
~150 mL DI H2O to dissolve KOH
~250 mL ETOH (95 %)

Dialysis Buffer (1 L)

150 mM	NaCl	8.8 g
15 mM	MgCl	15 mL 1M MgCl (or 3.04 g)
10 mM	Tris (pH 7.4)	10 mL 1 M Tris (pH 7.4)
	DI H2O	to 1 L

Stabilization Buffer for Microtubules

Stock A: 1 M PIPES (pH ~ 7.3)

33.5 g PIPES in 100 mL DI H2O

Stock B: 0.1 M EGTA (pH ~ 6.9)

1.9 g EGTA
15 mL DI H2O

pH with 1 M KOH
add DI H2O to 50 mL

Stabilization Buffer (10 mL)

1 mL	Stock A	(0.1 M PIPES)
0.1 mL	Stock B	(1 mM EGTA)
0.4 g	PEG 8000	(4 % Polyethylene Glycol)
13 mg	GTP (Na salt)	(2.5 mM GTP)
+/- 5 uL / mL	Triton X-100	(0.5 % Triton X-100)

PM2G Buffer (pH 6.9 1 L)

0.76 g	EGTA	(2 mM)
0.246 g	MgSO4.7H2O	(1 mM)
33.35 g	PIPES	(100 mM)
400 mL	DI H2O	
184.2 g	Glycerol	(2 M)

pH to 6.9 with 10 N NaOH
add DI H2O to 1 L
+ 0.1 % NP40 = 20 uL / mL (5 % NP40 stock)
+ PMSF = 1 uL / mL 0.2 M PMSF stock

PM Buffer (pH 6.9 100 mL)

3.34 g	PIPES	(100 mM)
0.025 g	MgSO4.7H2O	(1 mM)
1.47 g	CaCl2	(1 mM)
50 mL	DI H20	

pH to 6.9 with 10 N NaOH
add DI H2O to 100 mL

GSD Buffer (6 mL 4X)

2 mL	Glycerol
4 mL	10 % SDS
0.3 g	DTT
Some	Bromophenol Blue

Add DI H2O to 6 mL

TNT Buffer (6 L)

75 mL	2M Tris (pH 7.5)
59 g	NaCl
30 mL	Tween 20 (0.5 %)

Add DI H2O to 6 L

2 M Tris (pH 7.5 500 mL)

121 g	Tris Base
300 mL	DI H2O

pH with (lots) of HCl
Add DI H2O to 500 mL

PMSF (0.2 M 1000X 1 mL)
34.8 mg / mL in 95 % EtOH
Phenyl methyl sulfonyl fluoride (mw 174.2)

1 M Sodium Phosphate for blotting

1 L	1 M Di-basic Na2H(PO4)
500 mL	1 M Mono-basic NaH2(PO4)

Dilute to 20 mM for transfer

CsCl (density gradient)

74.2 g / 100 mL	CsCl	(1.546 g / mL)
37.1 g / 50 mL	CsCl	(1.546 g / mL)
61 g / 100 mL	CsCl	(1.45 g / mL)
27.7 g / 100 mL	CsCl	(1.2 g / mL)

Use DI H2O or Dialysis Buffer

Elisa III Buffer

15 mL	1 M NaCl	(150 mM NaCl)
1 mL	100 mM EDTA	(1 mM EDTA)
5 mL	1 M Tris (pH 7.4)	(50 mM Tris.HCl)
50 uL	Tween 20	(0.05 % Tween 20)

Adjust pH to 7.4
Add DI H2O to 100 mL
Add BSA to 0.1 %

1 M MOPS (pH 7.5 400 mL)

83.72 g	MOPS
250 mL	DI H2O

pH to 7.5 with 10 N NaOH
Add DI H2O to 400 mL

Fix for Antigen Staining

(PBS, 10 % sucrose, 3.7 % formaldehyde)

5 mL	10 X PBS
5 g	sucrose
5 mL	37 % formaldehyde

Add DI H2O to 50 mL

DAB for Westerns (30 mL)

3 mL	10 % imidazol
30 mg	DAB
30 uL	30 % H2O2 (Hydrogen peroxide)

Add DI H2O to 30 mL

1 M Tris (pH 7.4 500 mL)

60.5 g	Tris Base
350 mL	DI H2O

pH with HCl to pH 7.4 (~50 mL)

Add DI H2O to 500 mL

Completed Lysis Buffer for PI3K (100 mL)

4.57 mL	3 M NaCl	(137 mM NaCl)
2 mL	1 M Tris (pH 7.5)	(20 mM Tris)
0.1 mL	1 M MgCl2	(1 mM MgCl2)
0.1 mL	1 M CaCl2	(1 mM CaCl2)
10 mL	100 % glycerol	(10 % glycerol)
1 mL	NP-40	(1 % NP-40)

Add DI H2O to 100 mL
Store at 4C.
Just before use, add protease inhibitors.

Poly-lysine – Laminin Coating for cover slips

10 mL	poly-lysine
79 uL	laminin
3.78 mL	Sterile DI H2O

100 – 150 uL per cover slip (12 mm round)
1 hr at RT
Remove poly-lysine/laminin
Wash 2 X with Sterile DI H2O
Leave wet until use

1 X Semi-Dry Western Transfer Buffer

2.93 g	Glycine	(39 mM Glycine)
5.81 g	Tris-Base	(48 mM Tris)
0.375 g	SDS	
100 mL	DI H2O	
200 mL	Methanol	

Add DI H2O to 1 L

Mono Q buffers

QA (2 L: 20 mM Tris pH 7.7, 100 mM KCl, 1 mM MgCl2, 1 mM DTT)

40 mL	1 M Tris (pH 7.7)	
14.9 g	KCl	
0.406 g	MgCl2	
2 mL	1 M DTT	(1.54g DTT in 10 mL DI H2O = 1 M DTT)

Add DI H2O to 2 L

QA (alt)
1 X XB Salts (20 X Stock)
20 mM Tris-HCl (pH 7.7)

Degas before using on FPLC

QB (1 L QA + 0.9 M KCl)

1 L	QA
67.1 g	KCl

QB (alt)

1 L	QA (alt)	
141.6 g	KCl	(1.9 M KCl)

Degas before using on FPLC

TAE Buffer (50 X stock 1 L)

242 g	Tris Base
57.1 mL	Glacial Acetic Acid
100 mL	0.5 M EDTA (pH 8.0)

Add DI H2O to 1 L

PBS (1 X 100 mL)

0.8 g	NaCl	
0.2 g	KCl	
0.115 g	Na2HPO4	
0.02 g	KH2PO4	pH ~7.1-7.2

Filter Sterilize

Carbenicilin (100 X)

10 mg / mL Carbenicilin

Filter sterilize, then freeze/store 1 mL aliquots

20 X SSC

(3 M NaCl, 0.3 M Na3Citrate)

175.3 g	NaCl
88.2 g	Na3Citrate
800 mL	DIH2O

Adjust pH to 7.0 with 1 M HCl
Add DI H2O to 1 L

Kanamycin (1000 X)

30 mg / mL Kanamycin

Filter sterilize, then freeze/store 1 mL aliquots
1000 X stock for pET vectors
600 X for BactoBAC cells

IPTG (1 M Stock)

2.4 g	IPTG
10 mL	DI H2O

Filter sterilize, then freeze/store 1 mL aliquots
0.4 - 1.0 mM final working concentration (Stock is 1000 – 2500 X)

Lysis Buffer for sf9/baculo cells

20 mL	1 X TBS
20 uL	Triton X-100 (0.1 % Triton X-100)

2 X Sample Buffer (10 mL)

1 mL	1 M Tris (pH 6.8)
2 mL	20 % SDS
2 mL	100 % Glycerol
2 mL	1 % Bromophenol Blue
3 mL	DI H2O

Add 0.2 mL 1 M DTT prior to use

Buffer for S300 Column (1 L for CDC16)

20 mL	1 M Tris (pH 7.7)
7.45 g	KCl
1 mL	1 M DTT

Optional: add 1 mL of 1 M MgCl2
Add DI H2O to 1 L

Stacking Gel Solution

8 mL	Acrylamide:bis 37.5:1
7.5 mL	1 M Tris (pH 6.8)
0.3 mL	20 % SDS
44.4 mL	DI H2O

Makes 50 mL to be stored at 4C
For each 5 mL (one gel): add 50 uL 10 % APS (ammonium persulfate) and 5 uL TEMED

Western Strip Buffer (100 mL)

6.25 mL	1 M Tris (pH 6.7)
10 mL	20 % SDS
0.7 mL	BME (beta-Mercapto Ethanol)

Add DI H2O to 100 mL
Wash Blot 30 min at 50C

0.5 M Imidazole for bead elution (100 mL)

3.4 g	Imidazole
1 mL	0.5 M NaHPO4 (pH 6)
3.3 mL	1 M KCl

Add DI H2O to 100 mL

0.5 M NaHPO4 (pH 6 30 mL)

20 mL	0.5 M NaH2PO4 (pH ~4.3)
10 mL	0.5 M Na2HPO4 (pH ~7.2)

0.5 M NaH2PO4 (100 mL mw 120)

6 g	NaH2PO4
100 mL	DI H2O

0.5 M Na2HPO4 (100 ml mw 142)

7.1 g	Na2HPO4
100 mL	DI H2O

1 M DTT (10 mL Stock fw 154.2)

1.542 g	DTT
10 mL	DI H2O

Aliquot and store at -20C

20 X PBS (500 mL)

80 g	NaCl
20 g	KCl
11.5 g	Na2HPO4
2 g	KH2PO4 (pH 7.1-7.2)

Add DI H2O to 500 mL and filter sterilize

4 % paraformaldehyde (100 mL)

4 g	paraformaldehyde
100 mL	hot (60 – 65C) DI H2O

100 mM KxFe(CN)6 (100 mL)

4.2 g	K4Fe(CN)6
3.3 g	K3Fe(CN)6
100 uL	1 M MgCl2
100 mL	1 X PBS

6M Gu-HCl, 0.1 M Na Phosphate, 0.01 M Tris-HCl (pH 8) (50 mL)

28.7 g	GuHCl (fw 95.53)
10 mL	0.5 M NaHPO4 (pH 7.2)
0.5 mL	1 M Tris (pH 8.0)

Add DI H2O to 50 mL

Dialysis Buffer for APC urea Dialysis (1.5 L)

15 mL	1 M Tris (pH 7.4)	(10 mM Tris, pH 7.4)
26.5 g	NaCl	(300 mM NaCl)
0.3 g	MgCl2	(1 mM MgCl2)
1.5 mL	1 M DTT	(1 mM DTT)
3 mL	0.5 M EDTA	(1 mM EDTA)
0.22 g	CaCl2	(1 mM CaCl2)
0.15 g	BSA	(0.1 mg/mL BSA)
180 g	Urea	(2 M Urea)
	or	
90 g	Urea	(1 M Urea)

XB Extraction Buffer (20 X 1 X)

20 X Salts (2 M KCl, 20 mM MgCl2, 2 mM CaCl2)

1 X Salts (100 mM KCl, 1 mM MgCl2, 0.1 mM CaCl2)

1 X (1 X Salts, 10 mM Potassium HEPES (pH 7.7), 50 mM Sucrose)

20 X XB Salts Buffer (500 mL)

74.55 g	KCl
0.147 g	CaCl2-2H2O
2 g	MgCl2

Add DI H2O to 500 mL

PIN-1 Wash Buffer (20 mL)

1 mL	1 M Tris (pH 8.0)
2 mL	2 M NaCl
4 mL	0.5 M NaF
2 mL	100 % Glycerol
0.2 mL	Triton X-100
0.4 mL	0.5 M EDTA (pH 8.0)
1 uM	microcystin (okadaic acid)
1 X	protease inhibitors

Cyclohexamide (100 X Stock)

10 mg / mL	Cyclohexamide in DI H2O

Store frozen aliquots at -80C

Energy Mix (20 X 5 mL)

0.245 g	Creatine Phosphate
1 mL	100 mM ATP
100 uL	1 M MgCl2

Add DI H2O to 5 mL
Store in aliquots at -20C

EB (1 X 400 mL)

6.912 g	glycerophosphate	(80 mM)
3.044 g	EGTA	(20 mM)
1.22 g	MgCl2	(15 mM)
300 mL	DI H2O	

pH to 7.8 with KOH
Add DI H2O to 400 mL

Sperm Dilution Buffer (1 x 5 mL)

5 uL	1 M MgCl2	(1 mM MgCl2)
0.5 mL	1 M KCl	(100 mM KCl)
375 uL	2 M Sucrose	(150 mM Sucrose)
25 uL	1 M HEPES (pH 7.7)	(5 mM HEPES)
4.1 mL	DI H2O	

Fix for Nuclei (make fresh)

0.3 vol	37 % formaldehyde	
0.6 vol	80 % w/v glycerol	
0.1 vol	10 X MMR	
1 ug / mL	Hoechst dye bis benzimide	(10 mg / mL stock at -20C)

CSF-XB (100 mL)

5 mL	20 X XB Salts	(1 X Salts)
1 mL	1 M HEPES	(10 mM HEPES)
2.5 mL	2 M Sucrose	(50 mM Sucrose)
1 mL	0.5 M EGTA	(5 mM EGTA)

2 X – XN Buffer (100 mL)

10 mL	1 M HEPES (KOH, pH 7.0)	(100 mM HEPES)
10 mL	2 M Sucrose	(200 mM Sucrose)
7.5 mL	2 M NaCl	(150 mM NaCl)
25.4 mg	Spermidine-4HCl	(1 mM Spermidine)
10.44 mg	Spermine-4HCl	(0.3 mM Spermine)

Add DI H2O to 100 mL

G-PEM Buffer for rho tubulin

80 mM	PIPES (pH 6.8)
1 mM	MgCl2
1 mM	EGTA
1 mM	GTP
10 %	glycerol

0.5 M EGTA (100 mL pH 7.7)

19 g	EGTA
70 mL	DI H2O

pH to 7.7 with 10 N NaOH (~10-15 mL)
Add DI H2O to 100 mL

25 X MMR (Marc's Modified Ringers 2 L pH 7.8)

292.2 g	NaCl
7.45 g	KCl
10.15 g	MgCl2
14.7 g	CaCl2
1.8 g	EDTA
59.6 g	HEPES
1.2 L	DI H2O

pH to 7.8 with NaOH
Add DI H2O to 2 L

Nuclei Isolation Buffer A (100 mL)
(60 mM KCl, 15 mM NaCl, 0.15 mM Spermine, 0.5 mM Spermidine,
15 mM Tris (pH 7.4), 0.2 mM EDTA, 0.2 mM EGTA)

6 mL	1 M KCl
1.5 mL	1 M Tris (pH 7.4)
0.5 mL	3 M NaCl
40 uL	0.5 M EDTA
40 uL	0.5 M EGTA
150 uL	100 mM Spermine
250 uL	200 mM Spermidine

0.5 M Na pyrophosphate (dibasic) (20 mL pH 7.0)
2.21 g / 20 mL

pH to 7.0 with phosphoric acid

Stop Buffer (or Stabilization Buffer) SB (10 mL pH 7.0)
(50 mM NaF, 40 mM beta-glycerophosphate, 10 mM EDTA,
10 mM Na Pyrophosphate pH 7.0)

1 mL	0.5 M NaF
0.4 mL	1 M beta-glycerophosphate
0.2 mL	0.5 M EDTA
0.2 mL	0.5 M Na2HP2O7

2 X YT (per liter)

16 g	bactotryptone
10 g	bacto yeast
5 g	NaCl

pH to 7.0 with 5 N NaOH

Wash Buffer for GST Purification (200 mL pH 8.2)

10 mL	1 M Tris (pH 8.2)	(50 mM Tris)
2.96 g	KCl	(200 mM)
0.2 mL	1 M DTT	(1 mM DTT)

Add protease inhibitors before use

Lysis Buffer for GST Purification

Wash Buffer for GST Purification + 0.3 mM PMSF
+ 200 ug / mL lysozyme

10x Taq/Pfu Buffer
(from Teresita/Ethan August 2003)

200 mM Tris HCL
20 mM $MgSO_4$
100 mM KCl
100 mM $(NH4)_2\ SO_4$

1 mg/ml Nuclease-free BSA

FILTER

1% Triton X-100

AMINO ACIDS
(from Teresita/Ethan September 2001)

Amino Acid Mix (minus Methionine, Cysteine, Cystine)

- Assume **110g/ mole** for each amino acid.
- Want to add enough to make **2mM stock**

110g/ X = 1 M/ 0.002M (= 2 mM)

therefore X= 220 mg/ liter

- To make 50 ml
  - Add 11 mg of each amino acid **EXCEPT: METHIONINE, CYSTEINE, CYSTINE**
  - Bring to 40 ml with sterile water, pH to 7.0 with either HCL or NaOH add water to 50 ml in a sterile beaker/tube.

2 mM Methionine

11 mg Methionine
40 ml H_20
pH to 7.0
add H_20 to 50 ml

2 mM Cysteine/ Cystine

11 mg Cysteine
11 mg Cystine
40 ml H_20
pH to 7.0
H_20 to 50 ml

L-Amino Acids (SIGMA LAA-21) – 1 Kit

Translation Reaction Mix 5.0µl
(Teresita/Ethan May 2002)

Promega

Per Reaction (5 µl)

		IVT
TnT Retic lysate	2.5 µl	
Tnt Reaction buffer	0.2 µl	1) 5 µl TnT Mix + 1 µl DNA
RNA pol. SP6	0.1 µl	2) Incubate 1 hour @ 30 ^{0}C
AA (minus Met)	0.1 µl	
S^{35} Methionine	0.4 µl	
Rnasin	0.1 µl	
H_20	1.6 µl	

Homemade (5X E/T Buffer)

Per Reaction (5 µl)

Retic lysate*	2.5 µl
5X Reaction buffer	1 µl
RNA pol. SP6	0.1 µl
AA (**minus Met/ Cys**)	0.1 µl
S^{35} (**Met/Cys**)	0.4 µl
Rnasin	0.1 µl
H_20	0.8 µl
	5.0µl

*Promega Catalog # L4960

SIGMA C-9008 10 g
SIGMA H-1009 5 ml
SIGMA D-2650 100 ml

Solution B (Final concentration):

2.5 mM Luminol
0.4 mM p-Coumaric acid
100 mM Tris-HCl

Solution A	Solution B
10 ml	10 ml
5.5 µl	--------
---------	100 µl
---------	45 µl

Enhanced Chemiluminescence (ECL)2
(Teresita/ Licio/ Ethan May 2002)

Stock Solution:

250 mM Luminol (in DMSO)
90 mM p-Coumaric acid in (DMSO)
30% Hydrogen peroxide (H_20_2)
100 mM Tris-HCL (pH 8.5-9.0)
DMSO

Solution A (Final concentration)**:**

0.0165% H_2O_2
100 mM Tris-HCL

Volumes for 20 (final) ml of ECL Buffers

Stock Solutions

100mM Tris-HCL
30 % H_2O_2
250 mM Luminol
90 mM p-Coumaric Acid

FLUKA 09253 5 g

5X TnT Reaction Buffer

(Teresita/ Ethan May 2002)

Final Concentration:

50	mM	Potassium acetate
2	mM	Magnesium Chloride
0.05	mg/ml	Creatine kinase
1	mM	Creatine phosphate
1	mM	ATP
1	mM	CTP
1	mM	GTP
1	mM	UTP

Stock Solution:

1	M	Potassium Acetate	------	
40	mM	Magnesium Chloride	------	
1	Mg/ml	Creatine Kinase	ROCHE 127 566	100 mg
100	mM	Creatine phosphate	ROCHE 621 714	5 g
100	mM	ATP	SIGMA A-9062	100 mg
100	mM	CTP	SIGMA C-1631	100 mg
100	mM	GTP	SIGMA G-9002	100 mg
100	mM	UTP	SIGMA U-6875	100 mg