Stock Solutions

Revised by D Xiong
July 2007

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1 Kb DNA Ladder (Gibco BRL)

Working stock:
20 µl 1 µg/λ 1Kb DNA ladder (50 ng/ λ)
310 µl Tris EDTA pH 8.0 (TE)
70 µl 6X DNA load dye

_____
= 400 µl

Store at 4°C

Use ≥ 12 µl / lane

A.

Acidified Acetone

(0.024 M HCl in acetone)

198 µl 12.1 M HCl
100ml acetone

Store in glass bottle -20°C

Acid Urea - Running Buffer

5% acetic acid:
50 ml acetic acid
950 ml diH2O

Acid Urea – Pink Sample Buffer (2X)

1 ml acetic acid (10%)

4.8 g urea (8 M)
0.004 g Pyronin Y (0.04%)
800 µl β-mercaptoethanol (8%)

q.s. to 10 ml w/ diH20

Acid Urea – Transfer Buffer

0.7% acetic acid

Acrylamide : bis-acrylamid 30 : 0.8

500 ml ➔ 150 g 99.9% acrylamide (Bio-Rad) 161-0101
4 g bis N,N'-methylene-bis-acrylamide (Bio-Rad) 161-0201

Make sure it is all in solution!
Filter
Store in bottle + aluminum foil or dark bottle
➔ 4°C

Acrylamide: bis-acrylamide 60 : 0.4

250 g bottle of acrylamide (total vol. = 416.7 ml)

Add ˆ175 ml diH20
Stir o/n in dark

Add 1.67 g Bis & stir

Store room temp. in dark

Ammonium Persulfate (10%) – APS

1 g APS
10 ml diH2O

In 10 ml conical
Mix well, store 4°C ≤ 1 month

Ampicillin

Make 100 mg/ml stock in 50% ETOH

Make 10 ml:
1 g of ampicillin
10 ml 50% ETOH
Mix well

Aliquot into microtubes and store -20°C

C.

Ca-PO4 Transient Trasfection

2 M CaCl2 (from EM)

Filter and store at 4°C

ChIPs: Elution Buffer

Per ml:
200 µl 10% SDS (2%)
10 µl 1 M DTT (10 mM)
100 µl 1 M NaHCO3 (100 mM)
690 µl H2O

_____
= 1 ml

Prepare just prior to use

ChIP: Wash Buffer I

Per 10 ml:
250 µl 10% Triton X-100 (0.25%)
200 µl 0.5 M EDTA pH 8 (10 mM)
10 µl 0.5 M EGTA pH 8 (0.5 mM)
100 µl 1 M Hepes pH 7.4 (10 mM)
9.21 ml diH2O

Just prior to use, add :

100 µl 100mM PMSF
100 µl 100 X Microcystin
10 µl 1 mg/ml aprotinin/leupeptin
10 µl 1 mg/ml pepstatin

= 10 ml ➔ place on ice

ChIP: Lysis Buffer

Per 10 ml:
375 µl 4 M NaCl (150 mM)
250 µl 1 M Tris pH 7.5 (25 mM)
100 µl 0.5 M EDTA pH 8 (5 mM)
1 ml 10 % Triton X-100 (1%)
100 µl 10 % SDS (0.1%)
500 µl 10 % Na deoxychloric acid (0.5 %)
7.445 ml diH2O

Just prior to use, add :
100 µl 100mM PMSF
100 µl 100 X Microcystin
10 µl 1 mg/ml aprotinin/leupeptin
10 µl 1 mg/ml pepstatin

= 10 ml ➔ place on ice

ChIP: Wash Buffer II

Per 10 ml:
500 µl 4 N NaCl (200mM)
20 µl 0.5 M EDTA pH 8 (1 mM)
10 µl 0.5 M EGTA pH 8 (0.5 mM)
100 µl 1 M Hepes pH 7.4 (10 mM)
9.14 ml diH2O

Just prior to use, add :
100 µl 100mM PMSF
100 µl 100 X Microcystin
10 µl 1 mg/ml aprotinin/leupeptin
10 µl 1 mg/ml pepstatin

= 10 ml ➔ place on ice

Co-IP Buffer (from Blue Protein Book)

For 50 ml:
2.5 ml 1 M Tris-HCl pH 7.5 (50 mM Tris)
1.5 ml 0.5 M EGTA (15 mM)
1.7 ml 3 M NaCl (100mM)
500 λ 10 % Triton X-100 (0.1%)
43.8 ml diH2O

This can be stored at 4°C

Just prior to use, add :
1 mM PMSF
1 mM DTT
1 µg/ml aprotinin/leupeptin
1 µg/ml pepstatin

Coomassie Blue Stain

1 L:
500 ml MeOH (50%)
100 ml Glacial Acetic Acid (10%)
1 g Brilliant Blue (R – 250)
400 ml diH2O

In 1 L bottle, add 500 ml MeOH w/ stir bar, then add R-250 and mix till dissolved, then add the acetic acid followed by the water. Mix well and store @ room temp.

Brilliant Blue R Sigma B-0149

D.

Destain

40% H2O
50% MeOH
10% Acetic Acid

5 L:
2 L H2O
2.5 L MeOH
500 ml Acetic Acid

6X DNA load dye

In a 50 ml conical tube
To make 40 ml:
23.72 ml diH2O
16 ml 100% glycerol (40%)
0.1 g bromophenol blue (0.25%)

_____
= 40 ml

Wrap w/ parafilm, mix well & store at 4°C

For use: aliquot 1 ml & store a bench

Bromophenol blue Sigma B-8026

G.

G418

Make as 50 mg/ml in H2O

Store aliquots @ -20°C

Gel Destain

20 L:
1 L MeOH
1.5 L Acetic Acid

Glycerol Stocks (Bacteria)

100 ml:
65 ml 100% glycerol (65%)
10 ml 1 M MgSO4 (0.1 M)
2.5 ml 1 M Tris pH 8.0
22.5 ml diH2O
_____
= 100 ml

add together in 250 ml bottle
autoclave
use: add 800 µl of this to 400 µl of bacteria in a 2 ml tube & mix well
flash freeze in liq N2 or dry ice – ETOH bath
store - 80°C

GST – Recombinant Protein Lysis Buffer (1X PBS)

500 ml:
4.09 g NaCl (140 mM)
0.101 g HCl (2.7 mM)
0.6 g Na2HPO4 (10mM)
0.122 g KH2PO4 (1.8 mM)

Adjust pH to 7.3 w/ NaOH

Store @ 4°C

Guamidine – HCl Reversal Solution

4 M Guan-HCl 10 mM EDTA 0.5 M β-Me

H.

2X HEBS (250 ml) : for Ca-PO4 Transient Transfection

4.1 g NaCl (0.28 M final)
2.98 g HEPES (0.05 M final)
0.053 g Na2PO4 (1.5 mM final)

pH with 5 M NaOH, pH 7.05 – 7.12

filter @ store at 4°C

High Salt Extraction Buffer

10 ml:
200 µl 1 M HEPES pH 7.4 (20 mM)
840 µl 5 M NaCl (420 mM)
15 µl 1 M MgCl2 (1.5 mM)
5 µl 1 M DTT (0.5 mM)
8.82 ml diH2O
100 µl 100 mM PMSF (1 mM)
10 µl 1 mg/ml pepstatin (1 µg/ml)
10 µl 1 mg/ml aprotinin/leupeptin (1 µg/ml)

His-tagged Native Recombinant Protein Lysis Buffer

500 ml:
3 g NaH2PO4 (50 mM)
8.765 g NaCl (300 mM)
0.34 g Imidazole (10 mM)

Adjust pH to 8.0 with ION NaOH

Wrap in aluminum foil and store @ room temp.

His-tagged Native Recombinant Protein Wash Buffer

500 ml:
3 g NaH2PO4 (50 mM)
8.765 g NaCl (300 mM)

0.68 g Imidazole (20 mM)

Adjust pH to 8.0 with ION NaOH

Wrap in aluminum foil and store @ 4°C

His-tagged Native Recombinant Protein Elution Buffer

500 ml:
3 g NaH_2PO_4 (50 mM)
8.765 g NaCl (300 mM)
8.51 g Imidazole (250 mM)

Adjust pH to 8.0 with ION NaOH

Wrap in aluminum foil and store @ 4°C

Hydroxyurea

Make as 50 mg/ml in H_2O

Store in aliquots in -20°C

HPLC Solution – Poly Cation – Solution A

0.2 M $NaClO_4$ / 10 mM PO_3^{2-} (pH 6.5)

2.5 L :
70.23 g $NaClO_4$
3.55 g Na_2HPO_4 (dihase)

Mix to final vol. 2.0 L

Adjust pH to 6.5 with HCl

Bring final vol. to 2.5 L

HPLC Solution – Poly Cation – Solution B

0.6 M $NaClO_4$ / 10 mM PO_3^{2-} (pH 6.5)

2.5 L :

210.69 g NaClO4-
3.55 g Na2HPO4 (dihase)

Mix to final vol. 2.0 L

Adjust pH to 6.5 with HCl

Bring final vol. to 2.5 L

HPLC Solution – Poly Cation – Solution C

10% ACN

100 ml 100% ACN

900 ml diH2O

1 L

HPLC Solutions: C-8

Solution A:
5% ACN / 0.1% TFA
100 ml 100% ACN
2 ml 100% TFA
Q.s. to 2 L w/ H2O and FILTER

Solution B:
90% ACN / 0.1% TFA
1.8 L 100% ACN
2 ml 100% TFA
Q.s. to 2 L w/ H2O and FILTER

Solution C:
90% ACN
1.8 L 100% ACN
Q.s. to 2 L w/ H2O and FILTER

H2SO4 (0.4 N)

Molarity of 18 (stock) = 36 N (2 X Hs)

1 X 1/ml of 36 N H_2SO_4

99 ml of diH_2O

I.

IP Buffer (from Invitrogen)

50 ml:
2.5 ml 3 M NaCl (150 mM NaCl)
2.5 ml 1 M Tris HCl pH 8 (50 mM Tris HCl)
500 λ NP-4O (1%)
44.5 diH_2O

Immediately before we add 1 µg/ml popstatin, aprotinin/leupeptin, 1 mM EDTA and 1 mM PMSF

L.

LB Media

1 L:
10 g trypton
5 g yeast – extract
5 g NaCl

5 L:
50 g trypton
25 g yeast – extract
25 g NaCl

LB + Kam Plate (30 µg/ml)

800 ml will be enough for 30 plates (1 sleeve)

800 ml:
8 g tryptone
4 g yeast extract

4 g NaCl
12 g Agar

Auto clave: wait until media cools to 50°C before Kam
Add 2.4 ml of 10 mg/ml Kam stock (-20°C)
Pour 25 ml media per plate and stock
After cooling, replace in plastic sleeve
Label
Store @ 4°C

P.S. Add 4.8 ml of Kam per 500 ml of plates

LB + Amp Plate (100 µg/ml)

800 ml will be enough for 30 plates (1 sleeve)

800 ml:
8 g tryptone
4 g yeast extract
4 g NaCl
12 g Agar

Auto clave: wait until media cools to 50°C before Amp
Add 800 µl of 100 mg/ml Amp stock (-20°C)
Pour 25 ml media per plate and stock
After cooling, replace in plastic sleeve
Label
Store @ 4°C

Laemmli Buffer (10X)

10 L:
302.75 g Tris base
1441.5 g Glycine
100 g SDS (* wear mask)

5 L:
151 g Tris base
721 g Glycine
50 g SDS (* wear mask)

Add 500 ml H2O to bottom and stir before adding ingredients
Q.s. to 10 L and let stir on

Lysozyme 20mg/ml

20 mg/ml in H2O

Aliquot 500 λ in each 1.7 ml microfuge tube

Store at -20°C

M.

Mimosine

Sigma 100 mg bottle

MW = 198.2

40 mM stock:
100 mg + 12.61 ml diH2O (or media)
add 1 M NaOH until soluble

Store in aliquots at -20°C

N.

Nuclear Isolation Buffer

10 ml:
300 µl 5 M NaCl (150 mM)
100 µl 1 M HEPES pH 7.4 (10 mM)
15 µl 1 M MgCl2 (1.5 mM)
50 µl 2 M KCl (10 mM)
500 µl 10% NP-40 (0.5%) 4°C
5 µl 1 M DTT (0.5 mM)
100 µl 100 mM PMSF (1 mM)
10 µl 1 mg/ml pepstatin (1 µg/ml)
10 µl 1 mg/ml aprotinin/leupeptin (1 µg/ml)
8.9 ml diH2O

_____
= 10 ml

Nakatani Washing Buffer

50 ml :
1 ml 1 M Tris-HCl pH 8.0 (20 mM)
1.7 ml 3 M KCl (100 mM)
0.250 ml 1 M MgCl2 (5 mM)
20 λ 0.5 M EDTA pH 8.0 (0.2 mM)
5 ml 100% glycerol (10%)
50 λ 100% Tween-20 (0.1%)
35 λ 14.26 M 2-βMe (10mM)
50 λ 250 mM PMSF (DMSO) (0.25 mM)
41.9 ml diH20

* Add 2-βMe and PMSF immediately before use

2 L:
40 ml 1 M Tris-HCl pH 8.0 (20 mM)
68 ml 3 M KCl (100 mM)
10 ml 1 M MgCl2 (5 mM)
0.8 ml 0.5 M EDTA pH 8.0 (0.2 mM)
200 ml 100% glycerol (10%)

2 ml 100% Tween-20 (0.1%)
1.4 ml 14.26 M 2-βMe (10mM)
2 ml 250 mM PMSF (DM80) (0.25 mM)
1.676 L diH20

NO-40% lysis Extraction Buffer (Protein Extraction) from Dr. Jones' Lab : From Gerda

50 ml:
2 ml 0.5 M Tris-HCl pH8 (20 mM)
1.7 ml 3.0 M NaCl (100 mM)
100 λ 0.5 EDTA (1 mM EDTA)
2.5 ml 10% NP-40 (0.5%)
43.8 ml diH2O

10 ml:
400 λ 0.5 M Tris-HCl pH8 (20 mM)
340 λ 3.0 M NaCl (100 mM)
20 λ 0.5 EDTA (1 mM EDTA)
500 λ 10% NP-40 (0.5%)
8.74 ml diH2O

* Immediately before use add 1 µg/ml Apo, Leu/Pep and 1 mM PMSF (40 λ of 250 mM PMSF for 10 ml)

Nocodazole

Make up as 10 mg/ml in DMSO

Store in aliquots -20°C

P.

Phosphate Buffered Saline (PBS) – 10X

1 L:
80 g NaCl (1.5 M)
2 g KCl
14.4 g Na2HPO4
2.4 g KH2PO4

pH to 7.4 w/ HCl

Ponceau Red Stain

200 ml:
0.8 g Ponceau S (0.4%) – Sigma P – 3504
16 ml 100% TCA (8%)
4 ml Acetic Acid (2%)
^ 180 ml diH2O

1 L:
4 g Ponceau S (0.4%)
80 ml 100% TCA (8%)
20 ml Acetic Acid (2%)
~ 900 ml diH2O

Propidium Iodide

100 ml:
1 ml 1 M Tris pH 8.0 (10 mM)
0. 2 ml 5 M NaCl (10 mM)
0.5 ml 10 mg/ml propidium iodide (5 mg)
1 ml 1 mg/ml RNase A (1 mg)
93.7 ml diH2O

100 ml store in 4°C in foil

Good for ^ 1 month

10 X Proteinase K Buffer

Per ml:
100 µl 1 M Tris pH 7.5 (100 mM)
100 µl 0.5 M EDTA pH 8 (50 mM)
250 µl 10% SDS (2.5%)
550 µl diH2O

_____
1 ml

Store room temp

0.5 M PIPES pH 8.0

Dissolve in H2O

Add NaOH pellet one by one until the solution becomes clear

PHEM Buffer for IP

60 mM Pipes
25 mM Hepes
10 mM EGTA
2 mM MgCl2

pH to 6.9

R.

RIPA Buffer

10 ml:
300 µl 5 M NaCl (150 mM)
100 µl 10% SDS (0.1%)
500 µl 10% deoxycholic acid (0.5%) – make w/ H2O store in rm. temp
100 µl Triton X-100 (1%)
500 µl 1 M Tris pH 8 (50 mM)
5 µl 1 M DTT (0.5 mM)
100 µl 100 mM PMSF (1 mM)

10 µl 1 mg/ml pepstatin (1 µg/ml)
10 µl 1 mg/ml aprotinin/leupeptin (1 µg/ml)
8.375 ml H2O

Use fresh each time

S.

6X SDS Loading Dye (10 ml solution)

7 ml 0.5 Tris pH 6.8
3 ml glycerol (30% final)
1 g SDS (10% final)
1.2 mg bromophenol blue (0.012% final)

Filter 500 λ aliquot and store at -20°C

When ready to use, add DTT (0.6 M final concentration)

47 mg DTT / 500 λ loading dye

Once DTT is added, the dye lasts about 1 month

T.

Tris Buffered Saline (TBS) – 10X

1 L:
24.2 g Tris Base (200 mM)
80 g NaCl (1.5 M)

pH to 7.5 – 7.6 w/ conc. HCl

5 L:
121 g Tris Base (200 mM)
400 g NaCl (1.5 M)

pH to 7.5 – 7.6 w/ conc. HCl

5 L ➔ add 68 ml conc. HCl (12.2 M)

Towbin Buffer

1 L:
3.03 g Tris Base
14.4 g glycine
0.4 g SDS
_____
Add 100 ml MeOH

5 L:
15.15 g Tris Base
72 g glycine
2 g SDS
_____
Add 100 ml MeOH

TBS-T (Tris Buffered Saline + Tween-20)

1 L:
100 ml 10X TBS
900 ml diH2O
1 ml Polyoxyethylenescrbitan monolaurate (tween-20) Sigma P-1379

50X TBE (1 L)

10 X 1 L:
10.8 g Tris Base
5.5 g Boris Acid
4 ml 0.5 M EDTA pH8.0

10 X 5 L:
54 g Tris Base
27.5 g Boris Acid
20 ml 0.5 M EDTA pH8.0

50X TAE

1 L:

242 g Tris Base
100 ml 0.5 M EDTA pH 8.0

pH to 8.4 with glacial acetic acid (~57 ml)

Thymidine – Gibco BRL 16495-038 MW = 242

100 mM stock:
add 0.242 g to 10 ml diH2O (or media)
mix well and filter sterilize 0.45 µ
store in aliquots -20°C

Tris – EDTA (TE)

100 ml:
1 ml 1M Tris pH 8.0 (10 mM)
200 µl 0.5 M EDTA pH 8.0 (1 mM)
98.8 ml diH2O

Trichostatin A

Make up as 1 mM solution in DMSO

Store in -20°C aliquots

XYZ.

YPD Yeast media / Plates

Media:
add 50 g of YPD powder
bring volume w/ diH2O to 1 L
autoclave @ 121°C for 15 min

Plates:
add 50 g of YPD powder
50 g of Agar
Bring volume w/ diH2O to 1 L
autoclave @ 121°C for 15 min

allow media to cool down and then pour into plates ≈ 25 ml/plate
1 L makes ≈ 30 plates

DO: SD – Leu Plate / Media

Minimal SD Base – 26.7 g
C-TRP-Leu Mix – 0.64 g
L-Tryptophan – 10 mg = 0.01 g
Agar (for plates only) – 20 g
diH2O – 1 L

Autoclave for 15 min, cool down a little and pour plates

DO: C-TRP Yeast Media/Plates

Media:
add 26.7 g Minimal SD base
0.74 g –TRP DO supplement
bring volume w/ diH2O to 1 L
autoclave @ 121°C for 15 min

Plates:
add 26.7 minimal SD base
0.74 g –TRP DO
20 g Agar
Bring volume w/ diH2O to 1 L
autoclave @ 121°C for 15 min
for 500 ml of plates: add 75 λ of 10N NaOH then,
allow media to cool down and then pour into plates ≈ 25 ml/plate

TDO Plates/Media + 3AT (C-His-Leu-TRP)

1 L:
26.6 minimal SD base
0.62 g –His-Leu-TRP
20 g Agar
Bring volume w/ diH2O to 1 L

autoclave @ 121°C for 15 min

allow media to cool down to 65°C

once cooled add 7 ml of 1M 3-AT to 7 mM final concentration

2 X YPDA yeast / media

500 ml:
50 g YPD powder
15 mg Adenine
bring volume to 500 ml with diH2O

QPO Plates/Media (-His-Ade-Leu-TRP)

1 L: Makes 33 10cm plates and 14 15cm plates
add 26.6 minimal SD base
0.6 g -His-Ade-Leu-TRP
20 g Agar
Bring volume w/ diH2O to 1 L
autoclave @ 121°C for 15 min
pour ≈ 70-75 ml /plate
Before pouring allow media to cool down to ≈ 55°C ➔ cool down by slowly stirring

* 300 λ 5 M NROH before autoclave or 150 λ of 10 M NaOH / 1 L