Lab Buffers

making it easy....

Dankort Lab

2011

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Bacterial Reagents

Growth media (bacteria)

LB Medium

To make 1 liter, use 10 g tryptone, 5 g yeast extract, 10 g NaCl in distilled water (not milliQ pure). Adjust pH to 7.0. Sterilize by autoclaving.

LB Agar

Dispense 15 g per liter of agar directly into final vessel. Prepare LB medium as above and add to agar. NOTE: Agar will not go into solution until it is autoclaved (or boiled). If adding antibiotics, autoclave medium first and allow to cool until warm to the touch, then add the antibiotic. Dispense about 30 ml per plate. Allow plates to dry either at room temp overnight or 20 minutes in a laminar flow hood (lids removed). Store in original Petri plate bags, inverted, at 4°C for up to 2 weeks.

SOB Medium

Per liter add:
20 g Bacto-tryptone, 5g Yeast extrac 0.584 g NaCl , 0.186 g KCl
Mix components and adjust pH to 7.0 with NaOH and autoclave.

2 M Mg ++ stock

MgCl 2-6H2O 20.33 g
MgSO 4 -7H2O 24.65 g
Distilled water to 100 ml. Autoclave or filter sterilize.

2 M Glucose

Glucose 36.04 g
Distilled water to 100 ml. Filter sterilize.

For SOB Medium + magnesium: Add 1 ml of 2 M Mg ++ stock to 99 ml SOB Medium.

SOC Medium

Add 1 ml of 2 M Mg ++ stock and 1 ml of 2 M Glucose to 98 ml of SOB Medium.

IPTG : isopropyl thiogalactoside, or isopropyl beta-D-thiogalacto-pyranoside. (order from GoldBio.com).

1M solution. The formula weight is 238.3, so this is 0.238 g in 10 ml of water. Sterilize by filtration, then store in the freezer.

Xgal : 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside. (*Order from GoldBio*).

X-gal is very expensive and should be used carefully. It must be dissolved in DMSO (dimethyl sulfoxide) or dimethyl formamide (DMF; e.g., Sigma D8654), not water! Store at -20°C protected from light (i.e. wrapped in foil). Use a glass or polypropylene tube.

Drugs (selection, activation, inhibition)

Drugs used for Prokaryotic genetic selection

Ampicillin

Prepare a stock of 100 mg/ml in water. Sterilize by filtration. Store at -20°C but avoid repeated freeze/thaw cycles. Use at a final concentration of 100 µg/ml. To obtain this in 100ml of liquid (LB), add 100ul stock solution.

Kanamycin

Prepare a stock of 50 mg/ml in water. Sterilize by filtration. Store at -20°C but avoid repeated freeze/thaw cycles. The final concentration for LB liquid culture for growing plasmids is 50ug/ml. To obtain 50ng/ml in 100ml of LB, add 100ul stock solution.

Carbenicillin

Prepare a 100mg/ml stock in water. Dissolve 1 g of carbenicillin into 10 ml of ddH2O. Filter sterilize through a 0.22 µm filter, aliquot and store at -20°C.
Use at 1:1000 dilution in LB or LB-Agar.

Chloramphenicol

The frozen stock solutions of chloramphenicol are at 25mg/ml in 100% ethanol. The final concentration for LB liquid culture is 25mg/ml. To obtain this in 100ml of LB, add 100ul stock solution.

Spectinomycin

Dissolve 500 mg spectinomycin in 10ml to make a 50mg/ml stock solution. Sterilize by filtration and store at -20. Use at 50-100ug/ml meaning add 1-2ul of stock per 1ml of solution (i.e. a 500x-1000x stock)

Tetracycline

The frozen stock solutions of tetracycline are at 15mg/ml in ethanol. The final concentration for LB liquid culture is 15ug/ml. To obtain this in 100ml of LB, add 100ul stock solution.

Drugs used for Eukaryotic genetic selection

Gentamicin

10mg/ml in H20, store at -20°C. Working concentration is 20µg/ml and 80µg/ml for Mycoplasma selection

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The below drugs are given with suggested working concentrations given the diversity in resistance many cell lines display it is always best to set up a "kill-curve" centered around the suggest concentration. You will need at least 4 days (Blasticidin, puromycin) to 2 weeks (G418, hygromycin, zeocin)

Blasticidin

Sterile water is used to resuspend blaticidin to a final concentration of 5mg/ml. Solution a is filter sterilized and stored in 0.5ml aliquots at -80°C. Suggested selection is 5-15 µg/ml. The pH of the aqueous solution should not exceed 7.0 to prevent inactivation of Blasticidin.

G418 / geneticin

Dissolve in 500 mg** in 10ml of sterile PBS or DMEM, filter sterilized and stored in 0.5ml aliquots at -80°C. **check the activity of each batch. Often it is 750ug of active ingredient per 1000ug of weighed powder. Adjust accordingly. A general final concentration is anywhere from 50-500 µg/ml of G418.

Hygromycin

50mg/ml stock solution is made in sterile water, filter sterilized and stored in 0.5ml aliquots at -80°C. Typical working concentrations are 200-400 µg/ml i.e. 40ul/10ml of media.

Puromycin

Puromycin.Dihydrochloride stock solution is made in sterile water at 5mg/ml, filter sterilized and stored in 0.5ml aliquots at -80°C. A general final concentration is 2-4ug/ml.

Zeocin

Resuspend Zeocin in sterile water or HEPES buffer (pH 7.25) at a concentration of 100 mg/ml. Filter sterilize the solution using a 0.22µm sterile filter and aliquots are stored at -80°C for long term storage. Working concentration ~100ug/ml.
In most cells Zeocin appears to be cytostatic. Here drug sensitive cells become growth arrested but do not quickly die. The best way to select with zeocin is to split the cells several times in the presence of the drug. It is imperative to carry a mock line, i.e. in parellel a sister cell line lacking zeocin resistance.

Other Drugs

Ciprofloxacin

Ciprofloxacin HCl powder (Sigma 17850-5G-F) is stored at room temperature. Liquid stock solutions should be stored frozen (-20°C or less). Add the appropriate amount of powder to distilled deionized water while mixing. Mix completely, filter sterilized (0.2uM), and store in 1ml aliquots at -20°C. *NOTE: Adding water directly to the powder may result in insoluble mixtures that cannot be filtered without losing potency*.

Add Ciprofloxacin (from 5-25 µg.mL, most commonly 10 µg/mL) to media to treat cells seeded at a density of 10^4-10^5. Cells are re-seeded or sub-cultured with Ciprofloxacin containing media at 3 to 4 day intervals and are treated for 12- 20 days, after which, it should no longer be necessary to include Ciprofloxacin HCl in the media.

AP20187

AP20187 is a synthetic dimerizer that can be used to induce homodimerization of Fv domain-containing fusion proteins. AP20187 has no immunosuppressive activity and is non-toxic to cells. To date, AP20187 has only been tested *in vitro* and in mice. We do not yet know whether it crosses the blood-brain barrier in mice or whether it works in yeast or any other model organisms. AP20187 cannot be used to dimerize wild type FKBP domains. If you have already made constructs using wild type FKBP domains you must use the dimerizer AP1510 (4). We will continue to supply AP1510 for use *in vitro* upon request.

Reconstituting AP20187

AP20187 (molecular mass 1428.8 Da) is provided in lyophilized form which should be reconstituted as a concentrated stock in an organic solvent. We recommend dissolving the lyophilized material in absolute ethanol to make a 1 mM solution (e.g. dissolve 250 µg AP20187 in 175 µl ethanol). After adding the appropriate volume of ice-cold ethanol, seal and vortex periodically over a period of a few minutes to dissolve the compound. Keep on ice during dissolution to minimize evaporation.

Storage and handling of AP20187

Once dissolved, the stock solution can be kept at -20°C indefinitely, in a glass vial or a microfuge tube. Further dilutions in ethanol can be similarly stored. At the bench, solutions in ethanol should always be kept on ice, and opened for as short a time as possible, to prevent evaporation and consequent changes in concentration.

Using AP20187 *in vitro* Working concentrations of dimerizer can be obtained by adding compound directly from ethanol stocks, or by diluting serially in culture medium just before use. In the latter case we recommend that the highest concentration does not exceed 5 µM, to ensure complete solubility in the (aqueous) medium. In either case, the final concentration of ethanol in the medium added to mammalian cells should be kept below 0.5% (a 200-fold dilution of a 100% ethanol solution) to prevent detrimental effects of the solvent on the cells.

BrdU Treatment

B5002-sigma (link)

Dissolve 0.5 g in 45 ml of dH2O, add 5mL 10x PBS and filter sterilize with 22 µm filter. Dispense in 1mL aliquots and freeze at -80°C. Give 0.1 ml of this 10 mg/ml solution per mouse (1 mg/mouse). Detect with FITC-labelled BrdU (BD Pharmingen 556028)

4-Hydroxytamoxifen

Sigma: H6278 (link) Minimum 70% of Z isomer (remainder primarily E-isomer)
Molecular Weight: 387.51, 25mg/ml = 64.514mM

Note: 4-OHT directly activates ER-fusions, not tamoxifen. All tissue culture experiments should use 4HT and not tamoxifen. For experiments involving animals, use 4-OHT only for topical (ie skin) treatments.

For Tissue Culture expts: Resuspend 5mg in 12.9ml EtOH to give a 1.0mM solution.
For Topical treatment of Mice: We resuspend at 25mg/ml in DMSO for topical treatment. That is, if you order 50mg you would resuspend it in 2ml of DMSO.

Tamoxifen

Sigma: T5648 (link) Molecular Weight: 371.51

For Systemic treatment of Mice: 10mg/ml Tamoxifen is made by placing 0.4g tamoxifen in 40ml of sterile (by autoclave) sunflower oil (corn or peanut oil work well too) in a 50ml Falcon tube. Wrap in tinfoil to protect from the light and put on an end-over-end rotator over night at room temp.

Alternatively, tamoxifen can be dissolved in EtOH and then mixed with oil. The EtOH can be distilled off in a bath of boiling water.

Tamoxifen is administered daily by intraperitoneal (i.p.) injection for a maximum of 6 weeks at a dose of 1 mg/20 g body mass per day.

Mifepristone ≥98%

Sigma M8046 (link), Molecular Weight: 429.59, 25mg/ml = 58.195mM, 10mM=4.296mg. Mifepristone is soluble in EtOH to at least 20mg/ml =~46mM.

For Tissue Culture expts: Resuspend 10mg in 23.2ml EtOH to give a 1.0mM solution. Activation of PR fusions tends to max-out around 1uM.
For Topical treatment of Mice: We resuspend at 25mg/ml in DMSO for topical treatment. That is, if you order 50mg you would resuspend it in 2ml of DMSO.
For Systemic treatment of Mice: (*Michelle Lamarche?*)

D-luciferin

Xenogen XR-1001 (1mg solid @-20°C) (or equivalent - note this is very expensive so it is best to find the best price. GoldBio sells this)
stock: 30mg/ml in 1xPBS

Avertin

Resuspend 10g2,2,2,-tribromoethanol Aldrich (**T48402**) in 10ml Tertiary amyl alcohol (2-Methyl-2-butanol), Sigma-Aldrich (Fluka 19954)
Sterilized dilutant: (autoclave is best. alt: 0.22uM filtration)

50ul	1M	Tris (pH7.4)
25ul	0.5M	EDTA

1370 ul	5M	NaCl
48.75 ml		H2O

Mix 39.0 ml of dilutant with 1.0 ml Avertin. Mix well overnight at 4°C works well. Wrap in foil and store at 4°C

PD0325901

PD0325901, a generous gift of Pfizer Global Research and Development, was administered as a suspension in HPMT. HPMT was made by dissolving hydroxy-propyl-methylcellulose (Sigma) in water to 0.5% (w/v) and then adding Tween 80 to a final concentration of 0.2%(v/v). PD0325901 was suspended to 2.5 mg/ml on an end-over-end rotator and was made fresh once a week.

NVP-BEZ235-AN

For in vitro studies:

Preparation This salt is more soluble than the free base (NVP-BEZ235**-**NX**)** and stock solution can be made in 100% DMSO. We generally do a 10 mM stock solution that we keep in the fridge (4 C). As for the free base, in case the compound still not dissolved , try the following: either heat suspension using 85° C water bath or directly pass the (glass) tube a few time in a gaz flame**.** This compound is extremely stable and can stand this condition. After thawing, solutions may reprecipitate. Same procedure should then be applied.

Concentration range: *In vitro, the compound inhibit PI3Ka wthin the 50-100 nM range. In cells p-Akt inhibition IC50 is around 20 nM. A dose range from 10 to 250 nM for cellular systems is therefore advised.*

Preparation for in vivo studies:

Preparation As a salt (the correction factor for this batch is 1.37**), BEZ235 could be given (p.o. ie oral) either as a solution:

Dissolve dry powder in 1 volume of NMP (1-methyl-2-pyrrolidone : Fluka : #69118). After dissolution (if needed, in warm water), add 9 volumes of PEG300 (Fluka: # 81160). The final ratio is : NMP 10% / PEG300 90%. Once it is in solution in NMP/PEG30, treat animals in the next 30 minutes /1 hour.(It may precipitate after a longer period of time)

Or a suspension: simply resuspend the powder in 0.5% Methylcellulose

Dosing animals

Make the solution to be administered just before use (fresh each day)
Dose animal by the oral (p.o) route with an application volume of 10 mL/kg
In Harlan athymic nude mice, sensitive tumor models (such as U87MG) respond to NVP-BEZ235 at a dose of 25 to 45 mg/kg, p.o given 1qd (once per day)

The pharmacokinetic of the compound might be different in other strains (eg Balb/c) and therefore a pilot study with a DRF of 10 to 40 mg/kg is recommended.

Luciferin

Xenogen XR-1001 (1mg solid @-20°C) (or equivalent - note this is very expensive so it is best to find the best price. GoldBio sells this)
stock: 30mg/ml in 1xPBS

Buffers and salts

Tris Buffered Saline:

A versatile buffer that can be used for many applications. Often used for washing immunoblots and immunohistochemistry.

TBS-T

amnt	[conc]		[final]
120ml	5M	NaCl	150mM
80ml	1M	Tris-HCl [pH 7.5]	20mM
20ml	10%	Tween-20	0.05%
total to	4000ml		ddH_2O

10xTBS

	amnt		[final]
	175.32g	NaCl	150mM
	48.456g	Tris-HCl	20mM
total to	2000ml	ddH_2O	

pH to 7.5 with the addition of ~22ml 12M HCl

Phosphate Buffered Saline:

PBS has many uses because it is isotonic and non-toxic to cells. It can be used to dilute substances. The addition of divalent ions can cause precipitation of salts.

10XPBS

80g	NaCl
2g	KCl
14.4g	Na_2HPO_4
2.4g	KH_2PO_4

Make to 1000ml with ddH2O. The pH is ~6.8, but when diluted to 1x PBS it should change to 7.4.

1XPBS

8.00g	NaCl
0.20g	KCl
1.15g	$Na_2HPO_47(H_2O)$
2.00g	KH_2PO_4

Bring to 900ml with ddH_2O and pH to 7.2 before bringing final volume to 1000ml. If used in cell culturing, the solution can be dispensed into aliquots and sterilized by autoclaving (20 min, 121°C, liquid cycle)

NaOH

The preparation of 10 N NaOH involves a highly exothermic reaction, which can cause breakage of glass containers. Prepare this solution with extreme care in plastic beakers. To 800 ml of H2O, slowly add 400g of NaOH pellets, stirring continuously. As an added precaution, place the beaker on ice. When the pellets have dissolved completely, adjust the volume to 1 liter with H2O. Store the solution in a plastic container at room temperature. Sterilization is not necessary.

NaCl

To prepare 1 liter of a 5 M solution: Dissolve 292 g of NaCl in 800 ml of H 2O. Adjust the volume to 1 liter with H 2O. Dispense into aliquots and sterilize by autoclaving. Store the NaCl solution at room temperature.

EDTA stock

To prepare 1 liter, 0.5M EDTA pH 8.0: Add 186.1 g of disodium EDTA-2H 2O to 800 ml of H 2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (approx. 20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approx. 8.0 by the addition of NaOH. For tetrasodium EDTA, use 226.1 g of EDTA and adjust pH with HCl.

3M Sodium Acetate - pH 5.2

To prepare a 3 M solution: Dissolve 408.3 g of sodium acetate-3H2O in 800 ml of H 2O. Adjust the pH to 5.2 with glacial acetic acid. Adjust the volume to 1 liter with H2O. Dispense into aliquots and sterilize by autoclaving.

10M Ammonium Acetate

To make 100ml, add 77.08g to 10ml of ddH20 and stir. Bring to desired volume with ddH20 and stir with heat to get into solution. Solubility is increased with heat.

Miniprep Reagents:

Buffer P1 - Resuspension Buffer

50mM Tris-Cl, pH 8.0, 10mM EDTA, 100ug/mL RNase A
Storage condition - 4oC after adding RNase A
Prep - Dissolve 6.06g Tris base, 3.72g EDTA-2H20 in 800mL dH20. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with dH2O. Add 100mg RNase A per liter of P1.

Buffer P2 - Lysis Buffer

200mM NaOH, 1% SDS
Storage condition - RT
Dissolve 8.09g of NaOH pellets in 950mL dH2O, 50mL 20% SDS solution. The final volume should be 1 liter.

Buffer P3 - Neutralization Buffer.

3.0M potassium acetate, pH 5.5
Storage condition - 4oC or RT

Dissolve 294.5g potassium acetate in 500mL dH2O. Adjust the pH to 5.5 with glacial acetic acid (about 110mL). Adjust the volume to 1 liter with dH2O.

Plasmid Prep Reagents:

Solution 1

50mM Glucose, 25 mM Tris HCl [pH8.0], 10mM EDTA [pH 8.0]

Lysozyme

20mg/ml made fresh in solution 1 before use.

Solution 2

0.2M NaOH, 1%SDS made in ddH20. This should be made fresh at least monthly.

Solution 3

To make 100 ml: add 60ml 5M Potassium acetate, 28.5ml ddH2O and 11.5ml glacial acetic acid. It is convenient to keep this at 4°C until use

Immunohistochemistry Reagents

Gelatin
1ml Teleostein (fish) Gelatin ~45% in ddH2O (Sigma Cat# G7765)

BSA fraction V
insert description catalog number

serum
insert description catalog number

Sodium Citrate Antigen Retrieval
10 mM Sodium Citrate (pH 6.0, 100 mM stock)

Electrophoresis Buffers

DNA Electrophoreses

50x TAE

Prepare a 50x stock solution in 1 liter of H2O:
242 g of Tris base
57.1 ml of glacial acetic acid
100 ml of 0.5 M EDTA (pH 8.0)
The 1x working solution is 40 mM Tris-acetate/1 mM EDTA.

5X (or 10X) TBE

Prepare a 5x stock solution in 1 liter of H2O:

54 g of Tris base
27.5 g of boric acid
20 ml of 0.5 M EDTA (pH 8.0)

The pH of the concentrated stock buffer should be approx. 8.3. Some investigators prefer to use more concentrated stock solutions of TBE (10x as opposed to 5x). However, 5x stock solution is more stable because the solutes do not precipitate during storage. Passing the 5x or 10x buffer stocks through a 0.22µm filter can prevent or delay formation of precipitates.

10X DNA Loading Buffer

- 25% Ficoll, 100 mM Tris-HCl & EDTA, dye

12.5 g Ficoll-400
5.0 mL 1 M Tris-Cl, pH 7.4
10.0 mL 0.5 M EDTA

Bring to 50 mL with ddH2O, heating to 65°C to dissolve.

Add 25 mg of dye(s) of choice (Xylene cyanol and Orange G work well together).

dye	**0.5-1.5% agarose**	**2.0-3.0% agarose***	**CAT#**
Xylene cyanol (green/blue)	10'000-4000 bp	750-200 bp	Sigma X4126
Cresol Red	2000-1000 bp	200-125 bp	Sigma 114480
Bromophenol blue (drk blue)	500-400 bp	150-50 bp	Sigma B8026
Orange G	<100 bp	?	Sigma O3756

I recommend first making 50mL 10X loading buffer WITHOUT any marker dyes. Try to add the minimum amount of dye - too much is a bad thing. You can then add different combinations of dyes and dilute them as you wish to attain your preferred color intensity. This will yield enough loading buffer for your entire career, and the careers of your future postdocs and your postdocs' postdocs. You'll also have enough to experiment wildly with various dye combinations! Note Brohmophenol blue is particularly a pain for genotyping as it tends to run where your PCR product does.

6X DNA Loading Buffer

Add 15 ml glycerol.
Make up volume to 50 ml with distilled water (or preferably TE to prevent microbe growth and to stop all reactions).
Add 25 mg bromophenol blue and 25 mg xylene cyanol FF

Protein Electrophoreses

(See for background video http://www.sciencelauncher.com/SDS-PAGE.html)
(See link for %acrylamide choices
http://www3.bio-rad.com/pages/LSG/PGpop_Crit_SDS-PAGE.html)

4X Seperating Gel Buffer

92.06g Tris Base
10ml 20%SDS
bring total to 500ml ddH2O

pH to 8.8 with ~12ml of 12N HCl and store at room temp.

Stacking Gel Buffer

6.06g Tris Base
2ml 20% SDS
bring total to 100ml ddH2O

pH to 6.8 with ~12ml of 12N HCl and store at room temp. To make stacking gel add 5ml ddH2O, 1.5ml 30% acrylamide and 2.2ml stacking buffer. Polymerize with the addition of 100ml Ammonium persulfate and 25ml TEMED

4X Tris HCl/SDS pH 6.8

6.05g Tris Base in 40ml ddH2O

pH to 6.8 with 1N HCl and bring to a total volume of 100ml with ddH2Ostore. Filter through a 0.45mM and add 0.4g SDS. Store at 4°C.

2x SDS-PAGE Gel Loading Buffer

25ml 4X Tris HCl/SDS pH 6.8
20ml 100% Glycerol
4g SDS
1mg Bromophenol Blue
bring total to 100ml ddH2O

Store at room temp. Prior to use add 60ml of b-mercaptoethanol for each 1ml of loading buffer

6x SDS-PAGE Gel Loading Buffer

7ml 4X Tris HCl/SDS pH 6.8
3.6ml 100% Glycerol
1g SDS
0.93G DTT
1.2mg Bromophenol Blue

Store at room temp.

10xSDS-PAGE Electrophoresis Buffer

120g Tris Base

577.2g Glycine
40.0g SDS

Bring to 4L with the addition of ddH20 and store at room temp.
Never pH this solution.

Transfer Buffer

9.06g Tris Base
43.23g Glycine
500ml Methanol

Bring to 3L with the addition of ddH20.
Never pH this solution.

Ponceau S:

Ponceau S dye is used to make a stain for rapid reversible staining of protein bands on nitrocellulose or PVDF membranes (the ones we use) and also for staining proteins on nitrocellulose acetate membranes. The following are 2 common stains formulations:

0.1% Ponceau S (w/v) in 5% acetic acid

5ml glacial acetic acid
90ml deionized water
5mg (0.1%) Ponceau S powder

Stain can be reused multiple times.

The other stain solution is: 2% Ponceau S (w/v) in 30% TCA, 30% sulfosalicylic acid

Southern Buffers

250mM HCl (=21.5ml 11.6N HCl / 1000ml)
1.5M NaCl/ 0.5M NaOH (=20g NaOH, 87.6g NaCl / 1000ml

PreHyb buffer: [5xSSC, 5xDenhardt's*, 0.5% SDS, 10% Dextran Slulfate, 10ug/ml denatured salmon sperm DNA]

2xSSC Diluted from 20xSSC stock [175.3g NaCl, 88.2g NaCitrate in 800ml H2O adjust pH to 7.0 with NaOH bring to 1 liter]

100xDenhardt's 2g 2% Ficoll (type 400), 2g 2% polyvinylpyrrolidone (PVP-40), 2g 2% BSA (fraction V) bring to 100ml with H2O. Filter sterilize, aliquot and store at -20°C

Low Stringency wash buffer : [2xSSC] @ room temp
High Stringency wash buffer : [0.1xSSC, 0.1% SDS] prewarm 65-68°C
Hot Stripping buffer (>85°C 0.1%SDS).

Protein Lysis Buffers

Harsh Lysis Buffers

RIPA Lysis Buffer

	[final]
Na phosphate[pH 7.0]	10mM
NaCl	150mM
NP-40	1.0%
SDS	0.1%
Na Deoxycholate	1.0%
NaF	10mM
EDTA	2mM

Bring to a total of 500ml with ddH2O. This can be stored at 4°C for a month. Just prior to use add protease inhibitors (leupeptin and aprotinin) and NaV3O4 to a final concentration of 1µg/ml and 1mM respectively [reference MCB 8:3354-]

Moderate Lysis Buffers

PLCγ Lysis Buffer

			[final]
50ml	0.5M	HEPES [pH 7.5]	50mM
15ml	5M	NaCl	150mM
50ml	100%	Glycerol	10%
50ml	10%	Triton X-100	1%
2.5ml	0.2M	EGTA	1mM
0.75ml	1M	MgCl2	1.5mM
20ml	0.25M	NaF	10mM
12.5ml	0.4M	NaPyrophosphate	10mM

Bring to a total of 500ml with ddH2O. This can be stored at 4°C for a month. Just prior to use add protease inhibitors (leupeptin and aprotinin) and NaV3O4 to a final concentration of 1µg/ml and 1mM respectively

HNTG Buffer

20ml	0.5M	HEPES [pH 7.5]	20mM
15ml	5M	NaCl	150mM
50ml	100%	Glycerol	10%
5ml	10%	Triton X-100	0.1%

Bring to a total of 500ml with ddH2O. This can be stored at 4°C for a month.

Just prior to use add protease inhibitors (leupeptin and aprotinin) and NaV3O4 to a final concentration of 1µg/ml and 1mM respectively

Modified TNE Lysis Buffer

25ml	1M	Tris HCl [pH 8.0]	50mM
15ml	5M	NaCl	150mM
5ml	100%	NP-40	1.0%
20ml	0.25M	NaF	10mM
12.5ml	0.4M	NaPyrophosphate	10mM
2ml	0.5M	EDTA	2mM

Bring to a total of 200ml with ddH2O. This can be stored at 4°C for a month.
Just prior to use add protease inhibitors (leupeptin and aprotinin) and NaV3O4 to a final concentration of 1µg/ml and 1mM respectively

Mild Lysis Buffers

CHAPS Lysis Buffer

10ml	1M	Tris HCl [pH 8.0]	50mM
2ml	5M	NaCl	50mM
1.4g		CHAPS	0.7%
8ml	0.25M	NaF	10mM
5ml	0.4M	NaPyrophosphate	10mM
2ml	0.5M	EDTA	2mM

Bring to a total of 200ml with ddH2O. This can be stored at 4°C for a month.
Just prior to use add protease inhibitors (leupeptin and aprotinin) and NaV3O4 to a final concentration of 1µg/ml and 1mM respectively

Protease and Phosphatase inhibitors

Inhibitor	Protease/phosphatase Inhibited	Final concentration in lysis buffer	Stock (store at -20°C)
Aprotinin	Trypsin, Chymotrypsin, Plasmin	2 µg/ml	Dilute in water, 10 mg/ml.
Leupeptin	Lysosomal	5-10 µg/ml	Dilute in water, 10 mg/ml.
Pepstatin A	Aspartic proteases	1 µg/ml	Dilute in methanol, 1mM.
PMSF	Serine, Cysteine proteases	1mM	Dilute in ethanol. You can re-use the same aliquot.
EDTA	Metalloproteases that require Mg^{++} and Mn^{++}	5mM	Dilute in dH2O, 0.5M. Adjust pH to 8.0.
EGTA	Metalloproteases that require Ca^{++}	1mM	Dilute in dH2O, 0.5M. Adjust pH to 8.0.
Na Fluoride	Serine/Threonine phosphatases	5-10mM	Dilute in water.
Na pyrophophate	Serine/Threonine phosphatases	5-10mM	Dilute in water
Na Orthovanadate	Tyrosine phosphatases	1mM	Dilute in water** see below

200mM Sodium Orthovanadate preparation

Sigma: 450243-10G

Sodium orthovanadate (Na_3VO_4) must be activated for maximal inhibition of phosphotyrosyl-phosphatases. This procedure depolymerizes the vanadate, converting it into a more potent inhibitor of protein tyrosine phosphatases. Ref: Gordon, J., Methods Enzymol. 201: 477-482, 1991. Molar mass:183.908 g/mol

Generally I make ~100ml at a time. Note: do not permit great changes in volume during boiling; put a loose lid on the container to protect from evaporation. This needs to be done under the fume hood.

1- Prepare a 200 mM solution of sodium orthovanadate (3.678g in 150ml)
2- Adjust the pH to 10.0 using either 1 N NaOH or 1 N HCl. The starting pH of the sodium orthovanadate solution may vary with lots of the chemical. At pH 10.0 the solution will be yellow.
3- Boil the solution until it turns colorless (approximately 10 minutes).
4- Cool to room temperature.

5- Readjust the pH to 10.0 and repeat steps 3 and 4 until the solution remains colorless and the pH stabilizes at 10.0.
6- Store the activated sodium orthovanadate as aliquots at -80°C. Discard if the samples turn yellow.

Sodium Fluoride (Sodium NaF)

Sigma: S7920-100G
Molecular mass: 41.9887 g/mol
Prepare a 500 mM stock solution in ddH2O.
Dissolve 10.5g in 400ml of ddH2O.
Bring to 500ml with ddH2)
Store at room temperature

Sodium Pyrophosphate

(Sigma: 221368-100G)
$Na_4P_2O_7$: 265.90, $Na_4P_2O_7 \cdot 10H_2O$:446.06

Prepare a 400mM stock solution in ddH2O

Phosphatase inhibitor cocktail

For 50 mls
Add 1.9 g of EGTA to water and pH to 7.5 with vortex.
2.1 g Sodium Fluoride
2.23 g Tetrasodium Pyrophosphate
110 mg ß-Glycerophosphate
Mix well and sterile filter.
Make 100 aliquots of 0.5 ml and freeze at -80°C.

EGTA: 100mM = 38 mg/ml of water; pH to 7.5 with HCl
Sodium Fluoride: 1.0 M = 42 mg/ml
Tetrasodium Pyrophosphate: 100 mM = 44.6 mg/ml
ß-Glycerophosphate: 10 mM = 2.2 mg/ml

Ready-to-use cocktails of inhibitors from various suppliers are available but you can make your own cocktail.

Making EMEM for Adenovirus precipitation

Reagents: EMEM Sigma cat# M-0268, Purchase as 10x1L
Mix the contents of one bottle (9.6g) with 1L milliQ pure water making sure you have rinsed out the container. Add 2.2 g of Sodium Bicarbonate. Check pH to ensure it is 8.0.