STOCK SOLUTION RECIPIES:

Tris-HCl Buffer

10X Tris-HCl (0.5M Tris Base, pH7.6):

Trizma Base ---------- 61 g
Distilled water ---------- 1000 ml
Adjust pH 7.6 using concentrated HCl
Store this solution at room temperature. Dilute 1:10 with distilled water before use and adjust pH if necessary.

20X Tris-HCl (1M Tris Base, pH7.6):

Trizma Base ---------- 122 g
Distilled water ---------- 1000 ml
Adjust pH 7.6 using concentrated HCl
Store this solution at room temperature. Dilute 1:20 with distilled water before use and adjust pH if necessary.

10X Tris-HCl-Tween 20 (0.5M Tris Base, 0.5% Tween 20, pH7.6):

Trizma Base ---------- 61 g
Distilled water ---------- 1000 ml
Adjust pH 7.6 using concentrated HCl and then add 5 ml of Tween 20.
Store this solution at room temperature. Dilute 1:10 with distilled water before use and adjust pH if necessary.

Note: Tris-HCl Buffer is used for specific cases of immunohistochemical staining.

*** **OR you can use Tris Base to make Tris-HCl (note that Tris base is different from Trizma)**

Tris is a chemical with basic properties, having a pKa of 8.1. It can be used to buffer solutions from drastic pH changes, keeping them in the pH range of 7.0 to 9.0.

Make any Tris-HCl buffer in this pH range, at any molarity using these simple steps

1) Calculate Moles of Tris Base

   mol/L * L = moles needed

2) Calculate Mass of Tris Base

   Determine the mass of Tris base to weigh by multiplying the number of moles by the molecular weight (121.14 g/mol) of Tris.

   moles needed * g/mol = g

3) Dissolve Tris Base in Water

Dissolve the required mass of Tris into a volume of deionized water approximately 1/3 of the desired volume of buffer to be made.

4) Adjust the pH

   Using a pH meter, titrate the solution of Tris with 1M hydrochloric acid (HCl) until the correct pH is reached.

5) Bring to Volume

   Add the TrisHCl mixture to a volumetric flask of the desired volume and add deionized water as required to complete the solution.

0.2M Phosphate Buffer-4 Liters (pH 7.4):

17.66g Sodium Phosphate Monobasic
90.03g Sodium Phosphate Dibasic Heptahydrate
4 Liters ddH_2O
pH should be 7.4, if not adjust with 1.0N NaOH or 1.0N HCl
(3 Liters-13.25g Mono and 67.52g Dibasic)

0.1M Phosphate Buffer Saline (PBS)-8 Liters:

Prepare 4 liters of 0.2M phosphate buffer (see above recipe)
Add 72g NaCl (0.9% or 9g/liter)
Add 4 liters of ddH_2O
pH=7.4

*For practical purposes, you can also make 16 liters of PBS by first preparing 4 liters of 0.4M Phosphate Buffer. This concentration uses twice as much Monobasic and Dibasic since 0.4M versus 0.2M means the solution is twice as concentrated. But remember, you must then dilute this solution by adding 12 liters of water to make a total of 16 liters at a concentration of 0.1M. Also, since we are using 9gNaCl/liter, a total of 144g of NaCl will be used.

0.1M Phosphate Buffer-1 liter:

0.5 Liter of 0.2 M Phophate Buffer Stock
0.5 Liter of ddH2O

0.1M Phosphate Buffered Saline with Azide-1 liter (PBS*):

Prepare one liter of 0.1M Phosphate Buffer (see recipe above)
Add 9g of NaCl
Add 200mg of sodium azide (contents of 1 eppendorf vial)
Add 0.3 ml Triton-X
*Be sure to use protective clothing and a mask when handling sodium azide under hood!!

1% Paraformaldehyde 2% Glutaraldehyde

0.2M Stock Phosphate Buffer pH 7.4	500ml
Paraformaldehyde	10g
Glutaraldehyde (25% in water)	80ml
Distilledwater	QS to 1000ml

Dissolve the paraformaldehyde in about 400 ml of water. Heat this solution to 58 to 60 degrees C(do not allow the solution to get to hot!!). Add 1N NaOH drop by dropuntil the solution turns clear. Add the phosphate buffer stock and allow to cool, and then add the glutaraldehyde. Ph the solution to 7.4 and FILTER before use.

1% Paraformaldehyde 2% Glutaraldehyde (Hrp Fix)

0.2M Stock Phosphate Buffer pH 7.4	500ml
Paraformaldehyde	10g
Glutaraldehyde (25% in water)	80ml
Distilled water	QS to 1000ml

Dissolve the paraformaldehyde in about 400ml of water. Heat the solutionto 58 to 60 degrees C (do not allow the solution to get too hot!!). Add 1N NaOH drop by dropuntil the solution clears.Add phosphate buffer stock and allow the solution to cool. Add theglutaraldehyde and FILTER.

2% PARAFORMALDEHYDE

0.2M Stock Phosphate Buffer pH 7.4	500ml
Paraformaldehyde	20g
Distilled water	QS to 1000ml

Dissolve the paraformaldehyde in about 400ml of water. Heat the solutionto 58 to 60 degrees C(do not allow the solution to get too hot!!). Add 1N NaOH drop by dropuntil the solution clears.Add phophate buffer stock and allow the solution to cool. pH to 7.4 and FILTER.

4% PARAFORMALDEHYDE

0.2 M Stock Phosphate Buffer pH 7.4	500ml
Paraformaldehyde	40g
Distilled water	QS to 1000ml

Dissolve the paraformaldehyde in about 400ml of water. Heat the solution to 58 to 60 degrees C(do not allow the solution to get too hot!!) Add 1N NaOH to the paraformaldehyde solution drop by drop until the solution clears. Add phosphate buffer stock and allow to cool. pH to 7.4 and FILTER.

4% PARAFORMALDEHYDE 0.2% GLUTARALDEHYDE

0.2M Stock Phosphate Buffer pH 7.4	500ml

Paraformaldehyde	40g
Glutaraldehyde (25% in water)	8ml
Distilled water	QS to 1000ml

Dissolve the paraformaldehyde in about 400 ml of water. Heat the solutionto 58 to 60 C(do not allow the solution to get too hot!!). Add 1N NaOH drop by dropuntil the solution clears. Add phosphate buffer stock and allow the solution to cool. Add the glutaraldehyde. pH to 7.4 and FILTER.

Sucrose solution

10% 10gram in 90 ml 0.1 M PB
20% 20gram in 80 ml 0.1 M PB
30% 30gram in 70 ml 0.1 M PB

Acrylamide for separating gel (Acrylamide : BIS = 30 : 0.135)

Acrylamide	30.00 g
BIS	0.135 g

Make volume to 100 ml with MQ water. Keep in dark (Brown bottle)

Separating gel buffer (pH 8.8) (Final Conc.)

Tris	12.11 g	1 M
SDS	0.27 g	0.27%

Dissolve in 80 ml MQ water, adjust pH to 8.8, make the vol. to 100 ml

Acrylamide for stacking gel (Acrylamide : BIS = 29.2 : 0.8)

Acrylamide	29.2 g
BIS	0.8 g

Make volume to 100 ml with MQ water. Keep in dark (Brown bottle)

Stacking gel buffer (pH 6.8) (Final Conc.)

Tris	3.03	0.25 M
SDS	0.20 g	0.2%

Dissolve in 80 ml MQ water, adjust pH to 6.8, make the vol. to 100 ml

SDS-PAGE running buffer

Tris	9.0 g
Glycine	43.2 g
SDS	3.0 g

Dissolve in 3 L MQ water

SDS-sample buffer

Glycerol	10 ml
Tris	0.757 g
SDS	2.5 g
2-Mercaptoethanol	5.0 ml

Dissolve in 100 ml MQ water

Bromophenol blue (BPB) solution

Dissolve 0.1 g BPB in 100 ml 10% glycerol

Blotting solution A (Final conc.)

Tris	36.33 g	0.3 M
Methanol	200 ml	20%
SDS	0.20 g	0.02%

Make vol to 1000 ml with MQ water, keep at 4 ºC

Blotting solution B (Final conc.)

Tris	3.03 g	25 mM
Methanol	200 ml	20%
SDS	0.20 g	0.02%

Make vol to 1000 ml with MQ water, keep at 4 ºC

Blotting solution C (Final conc.)

Tris	3.03 g	25 mM
s-Amino-n-Caproic Acid	5.20 g	40 mM
Methanol	200 ml	20%
SDS	0.20 g	0.02%

Make vol to 1000 ml with MQ water, keep at 4 ºC

Ponceau 3S staining solution

Dissolve 0.1 g Ponceau 3S in 100 ml 1% Acetic acid

Coomassie brilliant blue (CBB) solution

Methanol	1500 ml
Acetic acid	300 ml
Coomassie brilliant blue-R250	3 g

Dissolve in 3000 ml MQ water

Destaining solution

Methanol	1100 ml
Acetic acid	300 ml

Dissolve in 3000 ml MQ water

Acrylamide solution for 1D-PAGE(Acrylamide : BIS = 28.38 : 1.62)

Acrylamide(High grade)	28.38 g
BIS	1.62 g

Make vol to 1000 ml with MQ water, keep in dark

Lysis buffer for Western Blot

Lysis buffer stock

1 ml Tris HCl, pH 7.4 (pre-made stock)
0.584g NaCl
49 ml dH2O

This stock can be kept for up to 2 weeks at 4°C

Lysis buffer for tissue prep
10 ml lysis buffer stock (see above)
1 protease inhibitor tablet
0.2 ml Phosphatase inhibitors (1 mM Na3VO4 and 5 mM NaF to block both tyrosine and serine/threonine kinases, see below)

100 ml (50x Na3VO4 and NaF stock solution)
50 mM Na3VO4 0.92gl
500 mM NaF 2.0995g
100 ml dH2O

0.02 N H_3PO_4
Add 0.48 ml H_3PO_4 (42 N, specific weight 1.87, 72%) to 1000 ml MQ water

0.02 N NaOH
Dissolve 0.8 g NaOH to 1000 ml MQ water.

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RIPA buffer (RadioImmunoPrecipitation Assay) buffer:
RIPA buffer contains the ionic detergent sodium deoxycholate as an active constituent and is particularly used for nuclear membrane disruption for nuclear extracts. A RIPA buffer gives low background but can denature kinases. It can also disrupt protein-protein interactions (and may therefore be problematic for immunoprecipitations/pull down assays).

50mM Tris HCl pH 8
150 mM NaCl
1% NP-40
0.5% sodium Deoxycholate
0.1% SDS

The 10% sodium deoxycholate stock solution (5 g into 50 ml) must be protected from light.
The 100 mM EDTA stock solution is made with 1.86 g into 40 ml H2O and then add NaOH to dissolve and adjust pH to 7.4. Finally, adjust the total volume to 50 ml). Store the buffer at 4°C.

Nonidet-P40 (NP-40) buffer:

20 mM Tris HCl pH 8
137 mM NaCl
10% glycerol
1% nonidet P-40
2 mM EDTA

Sodium orthovanadate preparation:

This needs to be done under the fume hood
• Prepare a 100 mM solution in double distilled water
• Set pH to 9.0 with HCl
• Boil until colorless
• Cool to room temperature
• Set pH to 9.0 again
• Boil again until colorless
• Repeat this cycle until the solution remains at pH 9.0 after boiling and cooling
• Bring up to the initial volume with water
• Store in aliquots at -20°C

Note: do not permit great changes in volume during boiling; put a loose lid on the container to protect from evaporation. Discard if the samples turn yellow.

TBS 10x (concentrated TBS)
24.23 g Trizma HCl
80.06 g NaCl
Mix in 800 ml ultra pure water.
pH to 7.6 with pure HCl.
Top up to 1 L.

TBST
For 1 L:
100 ml of TBS 10x
+ 900 ml ultra pure water
+ 1ml Tween20

Medium stripping buffer:
Make fresh stripping buffer:
15 g glycine
1 g SDS
10 ml Tween20
Set the pH to 2.2
make up to 1 L with ultrapure water

Harsh stripping buffer:
to be done under the fumehood
For 100 ml:

20 ml SDS 10%
12.5 ml Tris HCl pH 6.8 0.5M
67.5 ml ultra pure water
Add 0.8ml ß-mercaptoethanol under the fumehood.

Nuclear Fractionation Protocol Reagents
Buffer A –
10 mM HEPES,
1.5 mM MgCl2,
10 mM KCl,
0.5 mM DTT,
0.05% NP40 (or 0.05% Igepal or Tergitol)
pH 7.9

To prepare **250 ml stock of buffer A** –
HEPES: 1M = 238.3 g/L, therefore 10 mM = 0.59 g/250 ml
MgCl2: 1M = 203.3 g/L, therefore 1.5 mM = 0.076 g/250 ml
KCl: 1M = 74.5 g/L, therefore 10 mM = 0.187 g/250 ml
DTT: 1M = 154.2 g/L, therefore 0.5 mM = 0.019 g/250 ml
NP40 = 0.05%

Buffer B –
5 mM HEPES,
1.5 mM MgCl2,
0.2 mM EDTA,
0.5 mM DTT,
26% glycerol (v/v)
pH 7.9

To prepare **250 ml stock of buffer B –**
HEPES: 1M = 238.3 g/L, therefore 5 mM = 0.295 g/250 ml
MgCl2: 1M = 203.3 g/L, therefore 1.5 mM = 0.076 g/250 ml
EDTA: 1M = 372.2 g/L, therefore 0.2 mM = 0.0186 g/250 ml
DTT: 1M = 154.2 g/L, therefore 0.5 mM = 0.019 g/250 ml
26% Glycerol (v/v) = 65 ml

4.6 M NaCl
87.66 g/326 ml

TBS (Tris Buffered Saline) pH 7.6-7.8:
For 10 litres:
60.6 g TRIS HCl
13.9 g TRIS base

87.66 g NaCl
10 litres Ultra pure water (H2O)

TBS 0.025% Triton X-100:
For 1 litre:
250 µl Triton X-100
999.75 ml TBS
pH 7.6-7.8

1.6% H2O2 (Hydrogen Peroxide) in TBS:
For 400 ml:
6.4 ml H2O2 (GPR = 30% w/w)
393.6 ml TBS
pH 7.6-7.8

BS - Blocking serum in TBS (100ml):
NGS (10%) 10ml
BSA (2%) 2ml
Triton (0.4%) 4ml of 10% Triton

10% NS (Normal Serum) with 1% BSA (Bovine Serum Albumin, Fraction 5) in TBS:
For 1 ml:
100 µl NS
10 mg BSA
900 µl TBS
pH 7.6-7.8

Primary antibody made up in TBS with 1% BSA:
(Example is of primary antibody used at a dilution of 1:10)
For 0.1 ml:
100 µl Primary antibody
10 mg BSA
900 µl TBS
pH 7.6-7.8

Secondary biotinylated antibody made up in TBS with 1% BSA:
(Example is of secondary biotinylated antibody used at a dilution of 1:200)
For 1 ml:
5 µl Secondary biotinylated antibody
995 µl TBS
pH 7.6-7.8

ABC (Avidin-Biotin) complex in TBS:

(Example is of ABC complex, each part used at a dilution of 1:100)
For 1 ml:
10 µl Streptavidin
10 µl HRP (or AP)-Biotin
980 µl TBS
pH 7.6-7.8

Bicarbonate/carbonate coating buffer (100 mM):
3.03 g Na_2CO_3,
6.0 g $NaHCO_3$ (1 L distilled water)
pH 9.6,

PBS (500 ml):
1.16 g Na_2HPO_4,
0.1 g KCl,
0.1 g K_3PO_4,
4 g NaCl
(500 ml distilled water) pH 7.4