

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

$$\text{mol/L} * \text{L} = \text{moles needed}$$

- 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.

$$\text{moles needed} * \text{g/mol} = \text{g}$$

- 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 ddH₂O

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 ddH₂O

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 Phosphate 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
Distilled water	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 too hot!!). Add 1N NaOH drop by drop until 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 solution to 58 to 60 degrees C (do not allow the solution to get too hot!!). Add 1N NaOH drop by drop until the solution clears. Add phosphate buffer stock and allow the solution to cool. Add the glutaraldehyde 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 solution to 58 to 60 degrees C (do not allow the solution to get too hot!!). Add 1N NaOH drop by drop until the solution clears. Add phosphate 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
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Paraformaldehyde	40g
Glutaraldehyde (25% in water)	8ml
Distilled water	QS to 1000ml

Dissolve the paraformaldehyde in about 400 ml of water. Heat the solution to 58 to 60 C (do not allow the solution to get too hot!!). Add 1N NaOH drop by drop until 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 °C

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 °C

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 °C

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

Urea	4.8 g
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- 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 β -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 Na₂CO₃,

6.0 g NaHCO₃ (1 L distilled water)

pH 9.6,

PBS (500 ml):

1.16 g Na₂HPO₄,

0.1 g KCl,

0.1 g K₃PO₄,

4 g NaCl

(500 ml distilled water) pH 7.4