

## BUFFERS AND STOCK SOLUTIONS

### Cytoskeletal bound proteins Extract Buffer:

10 mM Tris, pH 7.4  
100 mM NaCl  
1 mM EDTA  
1 mM EGTA  
1 mM NaF  
20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>  
2 mM Na<sub>3</sub>VO<sub>4</sub>  
1% Triton X-100  
10% glycerol  
0.1% SDS  
0.5% deoxycholate

### Soluble protein buffer:

20 mM Tris-HCl, pH 7.5  
1 mM EGTA (Ca<sup>2+</sup> chelator)

### RIPA buffer (RadiolImmunoPrecipitation 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 H<sub>2</sub>O 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  $\beta$ -mercaptoethanol under the fumehood.

**Nuclear Fractionation Protocol Reagents**

**Buffer A** – 10 mM HEPES, 1.5 mM  $MgCl_2$ , 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

$MgCl_2$ : 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  $MgCl_2$ , 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

$MgCl_2$ : 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 (H<sub>2</sub>O)

**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% H<sub>2</sub>O<sub>2</sub> (Hydrogen Peroxide) in TBS:**

**For 400 ml:** | 6.4 ml H<sub>2</sub>O<sub>2</sub> (GPR = 30% w/w)  
| 393.6 ml TBS pH 7.6-7.8

**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<sub>2</sub>CO<sub>3</sub>, 6.0 g NaHCO<sub>3</sub> (1 L distilled water) pH 9.6,  
PBS: 1.16 g Na<sub>2</sub>HPO<sub>4</sub>, 0.1 g KCl, 0.1 g K<sub>3</sub>PO<sub>4</sub>, 4 g NaCl (500 ml distilled water) pH 7.4