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$_2$P$_2$O$_7$
2 mM Na$_4$VO$_4$
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$^{2+}$-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).

50 mM 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$_2$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

1. Prepare a 100 mM solution in double distilled water.
2. Set pH to 9.0 with HCl.
3. Boil until colorless.
4. Cool to room temperature.
5. Set pH to 9.0 again.
7. Repeat this cycle until the solution remains at pH 9.0 after boiling and cooling.
8. Bring up to the initial volume with water.
9. 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 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 (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 |

10% NS (Normal Serum) with 1% BSA (Bovine Serum Albumin, Fraction 5) in TBS:
For 1 ml:  
| 100 μl NS |
| 10 μg 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 μg 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: 1.16 g Na₂HPO₄, 0.1 g KCl, 0.1 g K₂PO₄, 4 g NaCl (500 ml distilled water) pH 7.4