Buffers and stock solutions

Recipes and procedures for various buffers.

Buffers and stock solutions

Collapse all

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 Na4P2O7
- 2 mM Na3VO4
- 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 (Ca2+ chelator)

RIPA buffer (radio immuno precipitation 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 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 Tris-Buffered Saline), 1 liter:

- 24 g Tris base (Formula weight: 121.1 g)
- 88 g NaCl (Formula weight: 58.4 g)
- Dissolve in 900 ml distilled water
- pH to 7.6 with 12N HCl
- Add distilled water to a final volume of 1 liter

For a 1X solution, mix 1 part of the 10X solution with 9 parts distilled water and pH to 7.6 again. The final molar concentrations of the 1X solution are 20 mM Tris and 150 mM NaCl.

An alternative recipe for Tris buffer combines Tris base and Tris-HCl. This avoids the large volume of potentially hazardous hydrochloric acid that is needed to neutralize a solution of Tris base alone.

TBS 10X alternative recipe (concentrated Tris-Buffered Saline), 1 liter:

- 24 g Tris HCl (Formula weight 157.6 g)
- 5.6 g Tris base (Formula weight 121.1 g)
- 88 g NaCl (Formula weight 58.4 g)
- Dissolve in 900 ml distilled water
- The pH of the solution should be about 7.6 at room temperature. If too basic, adjust to pH 7.6 with concentrated HCl, and if too acidic, adjust with concentrated NaOH
- Add distilled water to a final volume of 1 liter

For a 1X solution, mix 1 part 10X with 9 parts distilled water and pH to 7.6 again.

The final molar concentrations of the 1X solution are 20 mM Tris and 150 mM NaCl.

TBST (Tris-Buffered Saline, 0.1% Tween-20):

For 1 liter: 100 ml of TBS 10X + 900 ml distilled water + 1ml Tween-20

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 liter with double-distilled water

Harsh stripping buffer:

This needs to be done under the fume hood.

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:

10mM HEPES, 1.5 mM MgCl2, 10 mM KCl, 0.5 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%

4.6 M NaCl - 87.66 g/326 ml

Nuclear fractionation protocol reagents 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 0.025% Triton X-100:

For 1 L: 250 µl Triton X-100

- 1 liter 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 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 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: 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

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