abcam

Buffers and stock solutions for western blot

A collection of 18 recipes, buffers and stock solutions for western blot

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Recipes for western blot buffers and stock solutions

- RIPA buffer (radioimmunoprecipitation assay buffer)
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- Cytoskeletal bound protein extract buffer
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- Primary antibody made up in TBS with 1% BSA
- Secondary biotinylated antibody made up in TBS with 1% BSA
- ABC (avidin-biotin complex) in TBS
- Bicarbonate/carbonate coating buffer (100 mM)

RIPA buffer

RIPA buffer contains the ionic detergent sodium deoxycholate as an active constituent and is particularly useful 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 immunoprecipitation and pull-down assays).

- 50 mM Tris HCl, pH 8.0
- 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.

NP-40 buffer

- 20 mM Tris HCl
- pH 8.0 137 mM NaCl
- 10% glycerol
- 1% NP-40
- 2 mM EDTA

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 $Na_4P_2O_7$
- 2 mM Na_3VO
- 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

Sodium orthovanadate preparation

All procedures must be carried out under the fume hood.

1. Prepare a 100 mM sodium orthovanadate solution with 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
6. Boil again until colorless
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
10. Discard if the samples turn yellow

Avoid large changes in volume during boiling; put a loose lid on the container to protect from evaporation.

TBS 10X (concentrated Tris-buffered saline)

For 1 L

- 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 12 N HCl
- Add distilled water to a final volume of 1 L

For a 1X solution, mix 1 part of the 10X solution with 9 parts distilled water and adjust 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)

For 1 L
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

1. 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.
2. Add distilled water to a final volume of 1 L.
3. For a 1X solution, mix 1 part 10X with 9 parts distilled water and pH to 7.6 again.
4. 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 L
- 100 mL of TBS 10X
- 900 mL of distilled water
- 1 mL Tween 20

Medium stripping buffer

- 15 g glycine
- 1 g SDS
- 10 mL Tween 20

1. Adjust the volume to 800 mL with distilled water
2. Adjust pH to 2.2
3. Bring volume up to 1 L with distilled water

Harsh stripping buffer

This needs to be done under a fume hood.

For 100 mL
- 20 mL SDS 10%
- 12.5 mL Tris HCl, pH 6.8, 0.5 M
- 67.5 mL distilled water
- Add 0.8 mL β-mercaptoethanol under the fume hood

Nuclear fractionation protocol reagents buffer A

- 10 mM HEPES
- 1.5 mM $MgCl_2$
- 10 mM KCl
- 0.5 DTT
- 0.05% NP-40 (or 0.05% Igepal or Tergitol) pH 7.9

To prepare 250 mL stock of buffer A

- 0.59 g HEPES
- 0.076 g $MgCl_2$
- 0.187 g KCl
- 0.019 g DTT
- 0.05% NP-40

Nuclear fractionation protocol reagents 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

- 0.295 g HEPES
- 0.076 g $MgCl_2$
- 0.0186 g EDTA
- 0.019 g DTT
- 65 mL glycerol

TBS 0.025% Triton X-100

For 1 L

- 250 µL Triton X-100
- 1 L TBS, pH 7.6–7.8

1.6% H_2O_2 (hydrogen peroxide) in TBS

For 400 mL

- 6.4 mL H_2O_2 (GPR = 30% w/w)
- 393.6 mL 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, 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 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