

Solutions and reagents

Lysis buffers

These buffers may be stored at 4°C for several weeks or for up to a year aliquoted and stored at -20°C.

Nonidet-P40 (NP40) buffer

150 mM NaCl
1.0% NP-40 (possible to substitute with 0.1% Triton X-100)
50 mM Tris-HCl pH 8.0
Protease Inhibitors

RIPA buffer (Radio Immuno Precipitation Assay buffer)

150 mM NaCl
1.0% NP-40 or 0.1% Triton X-100
0.5% sodium deoxycholate
0.1% SDS (sodium dodecyl sulphate)
50 mM Tris-HCl pH 8.0
Protease Inhibitors

Tris-HCl buffer

20 mM Tris-HCl pH 7.5
Protease Inhibitors

Running, transfer, and blocking buffers

Laemmli 2X buffer / loading buffer

4% SDS
10% 2-mercaptoethanol
20% glycerol
0.004% bromophenol blue
0.125 M Tris-HCl

Check the pH and adjust pH to 6.8.

Running buffer (Tris-Glycine/SDS)

25 mM Tris base
190 mM glycine
0.1% SDS

Check the pH, which should be about pH 8.3. Adjust if necessary.

Transfer buffer (Wet)

25 mM Tris base
190 mM glycine
20% methanol

Check the pH, which should be about pH 8.3. Adjust if necessary.

For proteins larger than 80 kDa, we recommend that SDS is included at a final concentration of 0.1%.

Transfer buffer (Semi-dry)

48 mM Tris
39 mM glycine
20% methanol
0.04% SDS

Blocking buffer:

5% milk or BSA (bovine serum albumin)

Add to TBST buffer. Mix well and filter. Failure to filter can lead to “spotting” where tiny dark grains will contaminate the blot during color development.

Procedure

1. Sample lysis

a. Preparation of lysate from cell culture

1. Place the cell culture dish in ice and wash the cells with ice-cold PBS.
2. Aspirate the PBS, then add ice-cold lysis buffer (1 ml per 10^7 cells/100 mm dish/150 cm² flask; 0.5ml per 5×10^6 cells / 60 mm dish / 75 cm² flask).
3. Scrape adherent cells off the dish using a cold plastic cell scraper, then gently transfer the cell suspension into a pre-cooled microcentrifuge tube.
4. Maintain constant agitation for 30 min at 4°C.
5. Spin at 16,000 x g for 20 min in a 4°C pre-cooled centrifuge.
6. Gently remove the tube from the centrifuge and place on ice. Transfer the supernatant to a fresh tube kept on ice, and discard the pellet.

b. Preparation of lysate from tissues

1. Dissect the tissue of interest with clean tools, on ice preferably, and as quickly as possible to prevent degradation by proteases.
2. Place the tissue in round-bottom microcentrifuge tubes or Eppendorf tubes and immerse in liquid nitrogen to “snap freeze”. Store samples at -80°C for later use or keep on ice for immediate homogenization. For a ~5 mg piece of tissue, add ~300 µl lysis buffer rapidly to the tube, homogenize with an electric homogenizer, rinse the blade twice with another 2 X 300 µl lysis buffer, then maintain constant agitation (for example, on an orbital shaker) for 2 hr at 4°C.
3. Centrifuge for 20 min at 16,000 x g at 4°C in a microcentrifuge for 20 min. Gently remove the tubes from the centrifuge and place on ice. Transfer the supernatant to a fresh tube kept on ice. Discard the pellet.

2. Sample preparation

- 2.1. Remove a small volume (50 µl) of lysate to perform a protein assay. Determine the protein concentration for each cell lysate.
- 2.2. To the remaining volume of cell lysate, add an equal volume of 2 X Laemmli Sample Buffer.



We recommend to reduce and denature the sample using the following method unless the online antibody datasheet directs that non-reducing and non-denaturing conditions should be used:

2.3 To reduce and denature: Boil each cell lysate in sample buffer at 100°C for 5 min and aliquot. Store lysates at -20°C. Note: aliquot cell lysates (50- 100 µl) to avoid repeat freeze/thaw cycles.

2.4 Defrost tubes containing cell lysate at 37°C. Centrifuge at 16,000 x g in a microcentrifuge for 5 min.

3. Loading and running the gel

- 3.1. Load equal amounts of protein into the wells of the SDS-PAGE gel, along with molecular weight markers. Load 20- 30 µg of total protein from cell lysate or tissue homogenate, or 10-100 ng of purified protein.
- 3.2. Run the gel for 1-2 hr at 100 V.



This time and voltage may require some optimization. We recommend following the manufacturer's instructions. A reducing gel should be used unless non reducing conditions are recommended on the antibody datasheet.

Gel percentage will depend on the size of the protein:

4-40 kDa	20%
12-45 kDa	15%
10-70 kDa	12.5%
15-100 kDa	10%
25-200 kDa	8%

4. Transferring the protein from the gel to the membrane

Prepare the transfer stack as follows:



As a guideline, the transfer should be run for 1 to 2 hr at 100V.



The membrane can be either nitrocellulose or PVDF; each has its advantages. "Activate" PVDF with methanol for one minute and rinse with transfer buffer before preparing the stack. The time and voltage may require some optimization. We recommend following the manufacturer's instructions. Transfer to the membrane can be checked using Ponceau Red staining before the blocking step.

The membrane is ready for antibody staining.

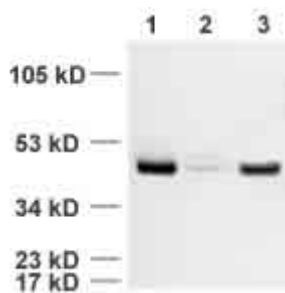
5. Antibody staining

- 5.1. Block the membrane for 1 hr at room temperature or overnight at 4°C using 5% blocking solution.
- 5.2. Incubate membrane with appropriate dilutions of primary antibody in 5% or 2% blocking solution overnight at 4°C or for 2 hrs at room temperature.
- 5.3. Wash the membrane in 3 washes of TBST, 5 min each.
- 5.4. Incubate the membrane with the recommended dilution of labeled secondary antibody in 5% blocking buffer in TBST at room temperature for 1 hr.
- 5.5. Wash the membrane in three washes of TBST, 5 min each, then rinse in TBS.
- 5.6. For signal development, follow the kit manufacturer's recommendations.
- 5.7. Remove excess reagent and cover the membrane in transparent plastic wrap.
- 5.8. Acquire image using darkroom development techniques for chemiluminescence, or normal image scanning methods for colorimetric detection.

Useful links:

View all [Abcam loading controls](#)

Example loading control: [ab8227 beta actin](#):



All lanes: beta Actin antibody - Loading Control (ab8227) at 1/5000 dilution

Lane 1: HeLa whole cell extract

Lane 2: Yeast cell extract

Lane 3: Mouse brain tissue lysate

View our list of available [positive control lysates](#) , blocking [peptides](#) and [positive control proteins](#).

View our more detailed [western blot guide](#).