alomone labs
empowering the spirit of science

Sample Preparation for Cell Lines

Clear protocols for preparing cells for western blot with Laemmli buffer or mild detergents.

Your sample preparation is critical for any western blot (WB) as it can affect the quality of your results. When it comes to using cell culture for WB analysis of your protein, the simplest method is to lyse them directly with electrophoresis (Laemmli) sample buffer. This method releases almost all cellular proteins into the buffer and are readily available for separation by standard SDS/PAGE methods. However, for some downstream applications (notably immunoprecipitation) you should refer to the Cell Line Preparation Using Mild Detergents.

It's worth paying attention to your sample preparation method, and choosing the right one based on your sample type, if you plan on producing reproducible and robust result. If you need sample preparation for tissues, take a look at our Sample Preparation Protocols for Tissues.

Lysate Preparation Using Laemmli Sample Buffer

This protocol refers to adherent cells but can be easily adapted to cells growing in suspension.

1. Wash the cell plate with ice-cold PBS. Repeat 3 times.
2. Place the plate on ice and add cold Sample Buffer (62.5 mM Tris (pH 6.8), 2% SDS, 10% glycerol, and 100 mM DTT) at a ratio of 5 × 10^6 cells/ml Sample Buffer.

Sample Buffer (Laemmli)

Reagent	Concentration
Tris (pH 6.8)	62.5 mM
SDS	2%
Glycerol	10%
Dithiothreitol (DTT)	100 mM

3. Scrape the dish with a cell scraper and collect the lysate into a microtube.
4. Boil the sample at 100°C for 5 minutes.
5. Sonicate the boiled sample for 5 seconds.
6. Centrifuge the sample at 14,000 rpm for 5 minutes at 4°C.
7. Transfer the supernatant to a clean tube and store at -80°C until further use.

Note: You can count cells can from a parallel plate by dislodging the cells with trypsin and counting them.

Cell Line Sample Preparation Using Mild Detergents

We recommend cell lysis with a mild detergent such as Triton X-100 or NP-40 for downstream applications like immunoprecipitation.

The protocol refers to lysis of adherent cells but can be easily adapted to cells growing in suspension.

1. Wash the cell plate with ice-cold PBS. Repeat 3 times.

2. Place the plate on ice and add ice-cold Lysis Buffer D at a ratio of 5×10^6 cells/ml Lysis Buffer D.

Lysis Buffer D

Reagent	Concentration
Tris (pH 7.6)	50 mM
Triton X-100	1%
EDTA (pH 8)	5 mM
NaCl	150 mM
Complete EDTA-free protease inhibitor cocktail (Roche)	

3. Scrape the dish with a cell scraper and collect the lysate into a microtube.

4. Rock the sample in the microtube for 30 minutes at 4°C.

5. Centrifuge the sample at 14,000 rpm for 10 minutes at 4°C.

6. Carefully transfer the clear supernatant to a clean microtube. You can store your sample at -20°C for several months or immediately mixed with Laemmli sample buffer.

Note: You can count cells from a parallel plate by dislodging the cells with trypsin and counting them.