Protocol

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Preparation of Protein Extracts for Western Blotting from Monolayer Cells

Introduction

Immunoblotting (also called Western Blotting) is used extensively in different protein studies aiming to characterize specific polypeptides, their interactions, and their modifications. This assay allows the detection of specific antigens by recognizing them with polyclonal or monoclonal antibodies. The first step, the preparation of the protein sample, is critical for successful immunoblotting, and requires lysing cells or tissue with an extraction buffer that will allow proteins to be solubilized for further analysis. The type of lysis buffer used is very important for appropriate solubilization of proteins, and the type chosen depends on the type of protein of interest. Here, we present a procedure for the preparation of a lysate from a monolayer of cells, using the common buffer RIPA, which contains the ionic detergent SDS, and allows for whole-cell extraction resulting in solubilization of cytoplasmic proteins that can then be analyzed through Western blotting.

Materials

- PBS (GoldBio Catalog # P-271), room temperature.
- Radio Immuno Precipitation Assay (RIPA) Buffer
- Phenylmethylsulfonyl Fluoride (PMSF) (GoldBio Catalog # P-470)
- Ice

Method

1. Grow cells to subconfluency in a Petri dish, remove culture medium and rinse cell monolayer with room temperature PBS.

Note: Perform the following steps on ice or at 4°C if using fresh, ice cold buffers.

2. Add 0.6 ml of Radio Immunoprecipitation Assay (RIPA) Buffer to the monolayer cells, gently rock for 15 minutes at 4°C, and remove cells with a cell scraper.

3. Transfer the resulting lysate to a microcentrifuge tube.

4. Wash the plate once with 0.3 ml of RIPA Buffer and combine with lysate.

5. Add 10 µl of 10 mg/ml PMSF and pass through a 21-gauge needle to shear DNA.

6. Incubate for 30-60 minutes on ice.

7. Centrifuge lysate at 10,000 x g for 10 minutes at 4°C. Transfer supernatant to new microcentrifuge tube.

8. For increased protein recovery, resuspend pellet in a small volume of RIPA, centrifuge and combine supernatant.

Tips

- RIPA Buffer does solubilize many cytoplasmic proteins. However, an insoluble fraction containing cytoskeleton and extracellular matrix proteins might also result. Thus, when choosing a lysis buffer, it is important to consider the type of protein that is being studied.
- For the preparation of cell lysates containing proteins that might have post-translational modifications, the lysis buffer should not contain any inhibitors, and should be performed in RIPA or NP-40 lysis buffers. These conditions will ensure these post-translational modifications are stable.
- Preparation of lysates containing cytoskeletal proteins, the use of Tris-Triton buffer is recommended.
- Preparation of lysates containing soluble cytoplasmic proteins, the use of 20mM Tris-HCl pH 7.5 buffer is recommended.
- Depending on the protein, phosphatase inhibitors might be needed.
- If an insoluble fraction results after lysing in RIPA Buffer, the pellet might be solubilized by boiling with dithiothreitol-containing Laemmli sample buffer.

Associated Products

- PBS tablets (GoldBio Catalog # P-271)
- PMSF (GoldBio Catalog # P-470)

References

Janes, K. A. (2015). An analysis of critical factors for quantitative immunoblotting. *Science Signaling,* 8(371). Doi:10.1126/scisignal.2005966.

Ngoka, L. C. (2008). Sample prep for proteomics of breast cancer: Proteomics and gene ontology reveal dramatic differences in protein solubilization preferences of radioimmunoprecipitation assay and urea lysis buffers. *Proteome Science,* 6(1), 30. Doi:10.1186/1477-5956-6-30.