Protocol

TD-P Revision 3.0

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Radio Immunoprecipitation Assay (RIPA) Cell Lysate Preparation

Introduction

Radioimmunoprecipitation Assay Buffer (RIPA) is used to lyse cells and tissues for use in radioimmunoprecipitation, protein assays, protein purification and other analytical procedures. Due to its ionic detergent composition, RIPA can disrupt nuclear membranes and solubilize cytoplasmic proteins, resulting in a high protein yield. This protocol outlines the preparation of RIPA buffer and its use with adherent cells, resulting in a protein lysate that can be used immediately or can be stored for future use.

Materials

- Adherent cell culture
- Tris-HCl (GoldBio Catalog # T-095)
- EDTA (GoldBio Catalog # E-210)
- EGTA (GoldBio Catalog # E-217)
- Triton X-100
- Sodium Deoxycholate (GoldBio Catalog # D-070)
- SDS
- NaCl
- dH_2O
- PMSF (GoldBio Catalog # P-470)
- PBS (GoldBio Catalog # P-271)
- ProBlock Gold™ Protease Inhibitor Cocktail (GoldBio Catalog # GB-108)

Preparation of RIPA lysis buffer:

- 10mM Tris-HCl, pH 8.0
- 1mM EDTA
- 0.5mM EGTA
- 1% Triton X-100
- 0.1% Sodium Deoxycholate
- 0.1% SDS
- 140mM NaCl
- Dilute with dH_2O
- This solution is stable at room temperature. Add 1mM PMSF immediately before use.

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Method

1. Prepare the RIPA Lysis Buffer. Add 1mM PSMF immediately before use.
2. Remove all media from the tissue culture dish.
3. Wash cells twice with PBS gently, pouring off excess into waste beaker.
4. Carefully soak up any extra PBS with an appropriate lab wipe.
5. Add 500 µl of RIPA Lysis Buffer to the culture dish.
6. Use a cell scraper to scrape cells from the bottom of the dish.
7. Pass cell lysate through pipette 20 times to form a homogeneous lysate.
8. Transfer lysate to 1.5 ml microcentrifuge tube.
9. Allow samples to stand for 5 minutes at 4°C.
10. Centrifuge the resulting mixture at 14,000 g for 15 minutes at 4°C to separate cell debris from protein.
11. Transfer supernatant to a new tube and store at -20°C.

Note: To ensure a protease-free supernatant if you perform protein purification, add one of our ProBlock Gold™ Protease Inhibitor Cocktail to your suspension, available in multiple formats for any application.

Associated Products

- Tris-HCl (GoldBio Catalog # T-095)
- EDTA (GoldBio Catalog # E-210)
- EGTA (GoldBio Catalog # E-217)
- Sodium Deoxycholate (GoldBio Catalog # D-070)
- PMSF (GoldBio Catalog # P-470)
- ProBlock Gold™ protease blocker (GoldBio Catalog # GB-108-2)
- PBS Tablets (GoldBio Catalog # P-271)

References

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

Monroy, F. (n.d.). RIPA Buffer Protocol. Retrieved April 26, 2018, from http://www2.nau.edu/fpm/documents/RIPAbuffer.pdf. Northern Arizona University.