Product Manual

Phosphoprotein Purification Kit

Catalog Number

AKR-106 **5 preps**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Protein phosphorylation-dephosphorylation is one of the major signaling mechanisms for modulating the functional properties of proteins involved in gene expression, cell adhesion, cell cycle, cell proliferation, and differentiation. Proteins can be phosphorylated by protein kinases on specific serine, threonine, or tyrosine residues. The utilization of anti-phosphoprotein antibodies in western blotting has become a commonly used tool for signal transduction research. Unfortunately, low levels of endogenous phosphoprotein in various cell lysates often can not be detected, even with high concentrations of antibody and long exposure times. With roughly 10% of cellular protein phosphorylated, a purification/enrichment of these phosphoproteins is a useful tool.

Cell Biolabs Phosphoprotein Purification Kit provides an efficient system for quick purification/enrichment of phosphoproteins from various samples. Phosphorylated proteins are affinity purified from lysates with a single-step purification matrix. The entire procedure takes about 4 hours. Each preparation can handle 2.5 mg of total lysate protein (approx. one confluent 100 mm dish).

Related Products

1. AKR-102: Phospho Antibody Stripping Solution
2. AKR-103: PhosphoBlocker™ Blocking Reagent (1L)
3. STA-415: ROCK Activity Immunoblot Kit
4. STA-416: 96-Well ROCK Activity Assay Kit

Kit Components

1. Phosphoprotein Enrichment Matrix (Part No. 210601): One tube – 0.6 mL of a 50% slurry.
2. Matrix Wash Solution (5X) (Part No. 210602): Two tubes – 1.5 mL each
3. Lysis Buffer (2X) (Part No. 210603): One bottle – 13.0 mL.
4. Loading Buffer (3X) (Part No. 210604): One bottle – 125.0 mL.
5. Elution Buffer (Part No. 210605): One bottle – 4.0 mL of 200 mM Na_2HPO_4, pH 8.0.

Materials Not Supplied

1. Phosphoprotein samples such as serum, plasma, tissue or cell lysate
2. BCA Protein Assay (Pierce)
3. 50 mL conical tubes
4. 1.5 or 2.0 mL microcentrifuge tubes
5. Shaker or rocker
6. Microcentrifuge (capable of 10,000 x g)
7. Cell culture centrifuge (capable of 1,500 x g)

Storage

Store all kit components at 4ºC.

Preparation of Reagents

- 1X Matrix Wash Solution: Mix the 5X Stock briefly and dilute to 1X in deionized water. Stir to homogeneity.
- 1X Lysis Buffer: Mix the 2X Stock briefly and dilute to 1X in deionized water. Just prior to usage, add desired protease inhibitors (e.g. 1 mM PMSF, 10 µg/mL leupeptin, 10 µg/mL aprotinin) and phosphatase inhibitors (e.g. 1 mM Na_3VO_4, 10 mM NaF). Stir to homogeneity.
- 1X Loading Buffer: Mix the 3X Stock briefly and dilute to 1X in deionized water. Stir to homogeneity.

Preparation of Samples

I. Adherent Cells

1. Culture cells to approximately 80-90% confluence.
2. Aspirate the culture media and wash twice with ice-cold PBS.
3. Completely remove the final PBS wash and add ice-cold 1X Lysis Buffer (See Preparation of Reagents) to the cells (0.5 - 1 mL per 100 mm tissue culture plate).
4. Place the culture plates on ice for 10-20 minutes.
5. Detach the cells from the plates by scraping with a cell scraper.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (14,000 x g at 4°C).
9. Collect the supernatant and store samples on ice for immediate use, or snap freeze and store at -80°C for future use.

II. Suspension Cells

1. Perform a cell count, and then pellet the cells by centrifugation.
2. Aspirate the culture media and wash twice with ice-cold PBS.
3. Completely remove the final PBS wash and add ice-cold 1X Lysis Buffer (See Preparation of Reagents) to the cell pellet (0.5 – 1 mL per 1 x 10^7 cells).
4. Lyse the cells by repeated pipetting.
5. Transfer the lysates to appropriate size tubes and place on ice.

6. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
7. Clear the lysates by centrifugation for 10 minutes (14,000 x g at 4°C).
8. Collect the supernatant and store samples on ice for immediate use, or snap freeze and store at -80°C for future use.

Assay Protocol

I. Preparation of Phosphoprotein Enrichment Matrix

1. Thoroughly resuspend the Phosphoprotein Enrichment Matrix by vortexing or titurating.
2. Transfer 100 µL of resuspended matrix slurry to a microcentrifuge tube.

   Note: 100 µL of resuspended matrix slurry is required per purification. If multiple purifications are to be done simultaneously, perform each independently.
3. Centrifuge the matrix for 5 minutes at 10,000 x g (microcentrifuge).
4. Aspirate and discard the supernatant, making sure not to disturb/remove the matrix pellet.
5. Add 1 mL of 1X Matrix Wash Solution to the pellet. Thoroughly resuspend the matrix pellet by vortexing or titurating.
6. Centrifuge the matrix for 5 minutes at 10,000 x g (microcentrifuge).
7. Repeat steps 4-6 once more.
8. Aspirate and discard the supernatant, making sure not to disturb/remove the matrix pellet.
9. Add 1 mL of 1X Loading Buffer to the pellet. Thoroughly resuspend the matrix pellet by vortexing or titurating.
10. Centrifuge the matrix for 5 minutes at 10,000 x g (microcentrifuge).
11. Repeat steps 8-10 once more.
12. After the last wash, pellet the beads and carefully remove all the supernatant.
13. Add 50 µL of 1X Loading Buffer to the pellet to form a 50% slurry.

II. Phosphoprotein Enrichment

1. Determine the protein concentration of the phosphoprotein sample (BCA Assay). Adjust the concentration to 1 mg/mL with 1X Lysis Buffer.
2. Transfer a maximum of 2.5 mg (or 2.5 mL at 1 mg/mL) of lysate sample to a 50 mL conical tube.
3. Perform a 10 fold dilution of the transferred sample with 1X Loading Buffer (yielding 0.1 mg/mL final). ***For example: Dilute a 2.5 mL sample to 25 mL total with 1X Loading Buffer.***

4. Thoroughly resuspend the washed Phosphoprotein Enrichment Matrix (step 13 above) by vortexing or titurating.
5. Quickly add 100 µL of resuspended matrix slurry to the phosphoprotein sample solution (from step 3).
6. Allow the matrix to mix with the protein sample at 4°C for 2 hours with agitation.
7. Centrifuge the matrix for 5 minutes at 1,500 x g (cell culture centrifuge).
8. Aspirate and discard the supernatant, making sure not to disturb/remove the matrix pellet.
9. Add 1 mL of 1X Loading Buffer to the pellet. Thoroughly resuspend the matrix pellet by vortexing or titurating. Transfer the mixture to a microcentrifuge tube.
10. Centrifuge the matrix for 5 minutes at 10,000 x g (microcentrifuge).
11. Aspirate and discard the supernatant, making sure not to disturb/remove the matrix pellet.
12. Add 1 mL of 1X Loading Buffer to the pellet. Thoroughly resuspend the matrix pellet by vortexing or titurating.
13. Repeat steps 10-12 two more times.
14. After the last wash, pellet the beads and carefully remove all the supernatant.
15. Elute captured phosphoproteins from the matrix by adding 0.5 mL of Elution Buffer.
16. Mix at room temperature for 10 minutes on an orbital shaker.
17. Collect the elution fraction by centrifugation for 5 minutes at 10,000 x g.
18. Carefully remove the elution supernatant.
19. If desired, final elution may then be concentrated with a centrifugal concentrator.

Example of Results

The following figures demonstrate typical purification results. One should use the data below for reference only. This data should not be used to interpret actual results.

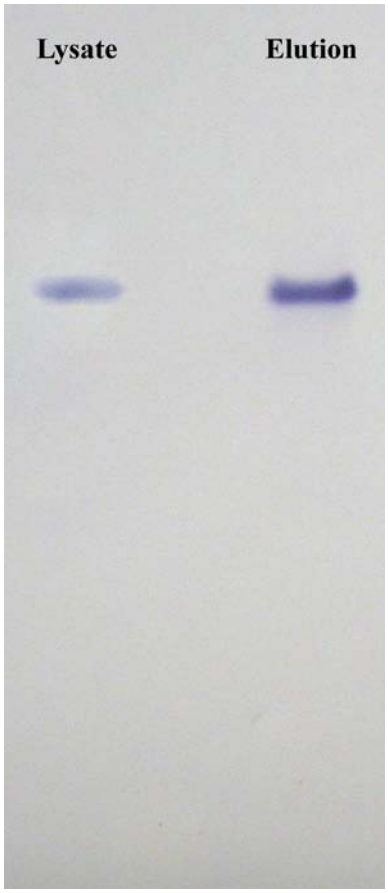

Figure 1: Enrichment of p-ERK. HeLa cell lysate was incubated with Phosphoprotein Enrichment Matrix according to the Assay Instructions. p-ERK enrichment was demonstrated by immunoblot.

References

1. Hunter T (2000) *Cell* **100**, 113–127.
2. Manning G, Whyte DB, Martinez R, Hunter T and Sudarsanam S (2002) *Science* **298**, 1912–1934.
3. Guerrera IC, Predic-Atkinson J, Kleiner O, Soskic V and Godovac-Zimmermann J (2005) *J Proteome Res* **4**, 1545–1553.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's

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