I. Introduction

This protocol is provided for the simple and rapid capture of protein-protein complexes from whole cell extracts using the **Capturem IP & Co-IP Kit** (Cat. No. 635721). The kit contains specially designed Protein A columns using our novel Capturem technology for efficient capture of antibody-bound target protein complexes. The included buffer set provides ease of use and rapid isolation of immunoprecipitated protein complexes allowing faster downstream analysis.

II. Materials and Reagents

A. Included

Box 1:

- 12 Capturem IP & Co-IP Columns

Box 2:

- Lysis/Equilibration Buffer (12 ml)
- Wash Buffer (2 ml)
- Elution Buffer (1 ml)
- Protease Inhibitor Cocktail (100X) (100 µl)

NOTE: The components of Box 2 are shipped on blue ice and should be stored at 4ºC. Prior to starting the protocol, bring the buffers and protease inhibitor up to room temperature.

B. Not Included

- Collection tubes

  Each purification will require three additional standard 2-ml collection tubes, with or without caps. These tubes should be used throughout the protocol to collect flowthrough samples that will be saved for western blot analysis.
- Neutralization buffer: 1 M Tris, pH 8
- Phosphate-buffered saline (PBS)
- Antibodies

  Preferably a polyclonal antibody for the immunoprecipitation of the protein of interest and a monoclonal antibody (against the same protein) from a different species as the primary antibody for detection. A secondary antibody (against the primary antibody) conjugated to a detection reagent is also needed.

III. Sample Preparation

1. Before starting the lysis protocol, remove the media from the cells and wash with cold PBS once. Prepare the Lysis/Equilibration Buffer by adding the appropriate amount of the Protease Inhibitor Cocktail to yield a 1X final concentration of inhibitors.

NOTE: Additional inhibitors for phosphatases such as sodium orthovanadate (not provided) may also be required depending on the downstream applications.

2. Add 200 µl Lysis/Equilibration Buffer per 1 x 10^6 cells. Incubate on ice for 15 min. Collect and centrifuge lysates at 17,000*g* for 10 min at 4°C. Remove and keep supernatant.

   EXAMPLE: A pellet consisting of 3 x 10^6 cells should be resuspended in 600 µl of Lysis/Equilibration Buffer containing 6 µl of Protease Inhibitor Cocktail.

3. Incubate the recommended amount of antibody with clarified cell lysates for up to 20 min at room temperature or 1 hr at 4°C.

IV. Immunoprecipitation

1. Add 100 µl Lysis/Equilibration Buffer to a spin column, placed in the provided collection tube, to equilibrate the column. Centrifuge at 1,000*g* for 1 min at room temperature. Remove the flowthrough and discard along with the provided collection tube, then place the column in a new collection tube (supplied by the user—see Section II.B).

2. Load 100–800 µl sample (pre-incubated with antibody) onto the equilibrated spin column. Centrifuge at 1,000*g* for 1 min at room temperature. Save the collection tube containing the sample flowthrough for protein analysis and transfer the spin column to a new collection tube (supplied by the user).

   NOTE: The flowthrough may be reloaded onto the same spin column in order to maximize the binding capacity of the membrane.

3. Add 100 µl Wash Buffer to the spin column. Centrifuge at 1,000*g* for 1 min at room temperature. Save the collection tube containing the wash flowthrough for protein analysis and transfer the spin column to a new collection tube (supplied by the user).

4. Before inserting the spin column into a new collection tube for elution, add 3–5 µl neutralization buffer (volume should be 1/10^{th} of the Elution Buffer volume to be used) to the collection tube. Then insert the column into the collection tube and add 30–50 µl Elution Buffer to the column. Centrifuge at 1,000*g* for 1 min at room temperature and vortex the contents of the collection tube to mix your eluted antibody-protein complex with the neutralization buffer in the tube. The eluted sample is now ready for analysis.

   NOTE: ≥80% of your antibody-protein complex can be eluted with 20 µl of Elution Buffer.

5. Analyze the samples using SDS-PAGE and western blot.

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