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I. Introduction

The following protocol uses **Anti-DYKDDDDK Beads** (Cat. No. 635686), the **Immunoprecipitation Buffer Set** (Cat. No. 635687), and **ProteoGuard EDTA-Free Protease Inhibitor Cocktail** (Cat. No. 635673) to purify or immunoprecipitate DYKDDDDK-tagged fusion proteins from cell lysates. These reagents are sufficient for sample preparation and immunoprecipitation of up to 30 reactions.

II. Sample Preparation and Immunoprecipitation

A. Materials Required

- 1X PBS
- Immunoprecipitation Buffer Set (Cat. No. 635687)
 - Lysis Buffer (100 ml)
 - Wash Buffer (2 x 125 ml)
- Anti-DYKDDDDK Beads (1 ml; Cat. No. 635686)
- ProteoGuard EDTA-Free Protease Inhibitor Cocktail (10 x 100 µl; Cat. No. 635673)

NOTE: At the time of your experiment, add 10 µl of 100X ProteoGuard EDTA-Free Protease Inhibitor Cocktail per ml of lysis buffer prior to lysing cells to yield a 1X final concentration of inhibitors.

B. Protocol: Preparation of Mammalian Cell Lysate

1. Wash a 10 cm dish containing adherent cells twice with PBS. Remove all residual PBS. For suspension cells, transfer 1×10^7 – 2×10^7 cells to a 15 ml conical tube. Wash twice with 10 ml PBS.
2. Add 2 ml Lysis Buffer per plate. Incubate for 15–30 min at 4°C on a rocking platform.
3. Collect cell lysate in a 2 ml tube.
4. Alternatively, you may disrupt cells by repeatedly passing the sample through a 21-gauge needle.
5. Clear the lysate by centrifuging at 12,000 rpm for 30 min at 4°C. Collect supernatant.

C. Protocol: Immunoprecipitation

1. Transfer 1.2 ml cleared lysate to a microcentrifuge tube. Be careful not to transfer any precipitated material as this can cause nonspecific background. If necessary, reclarify the lysate by centrifugation.
2. Transfer 30 μ l of Anti-DYKDDDDK Beads to a separate microcentrifuge tube and wash as follows:
 - a. Pulse at full speed, rotate (twist) tubes 180° in centrifuge slots, pulse again, and discard supernatant.
 - b. Resuspend bead pellet in 60 μ l of Wash Buffer, repeat Step 2.a, and resuspend in 30 μ l of Lysis Buffer.
3. Add washed, resuspended beads to cleared lysate.
4. Incubate for 1 hr to overnight at 4°C on a rotating apparatus.
5. Collect immunoprecipitates by centrifugation according to one of the following methods:
 - a. Recommended—Centrifuge beads at 3,000 rpm for 2 min, rotate (twist) tubes 180° in centrifuge slots, and centrifuge again for 2 min.
 - b. Quick method— pulse the microcentrifuge to full speed and then release. Rotate (twist) tubes 180° in centrifuge slots, and pulse to full speed again. Do not over-centrifuge as this may damage the beads.
6. Remove immunodepleted supernatant. Remove as much of the supernatant as possible without disturbing the bead pellet.
7. Wash three times with 1 ml Wash Buffer. If background is observed, the wash buffer can be supplemented with higher salt (for example, up to 1 M NaCl) or detergents such as Tween-20 or Triton X-100. Acceptable concentrations will need to be determined empirically.
8. Discard supernatant from final wash and resuspend pellet in 25–50 μ l 2X SDS sample buffer. Boil samples for 5 min and spin down.
9. Load 10–15 μ l of supernatant on an SDS/polyacrylamide gel. Continue with Western blotting as described in the next section.

III. Western Blotting

A. Materials Required

- 1X PBS (Alternatively, you may substitute Tris-based buffers.)
- 2X SDS sample buffer (see Sambrook *et al.*, 1989)
- Wash buffer (PBS or TBS containing 0.2% Tween-20)
- Blocking buffer (5% nonfat dry milk in wash buffer)
- Primary antibody
- Horseradish peroxidase (HRP)-conjugated secondary antibody
- HRP chemiluminescent detection system

B. Protocol: Western Blotting of Immunoprecipitated Protein

NOTE: Optimal dilutions and incubation times may vary with individual systems and must be determined empirically.

1. Transfer proteins from the gel (Section III.C, Step 8) to a nitrocellulose membrane using standard techniques.
2. Add 10 ml blocking buffer and incubate the membrane for 1 hr at room temperature with gentle rocking. Alternatively, block the membrane overnight at 4°C with rocking.
3. Dilute the primary antibody in 10 ml of blocking buffer according to the specifications on the Certificate of Analysis (or dilute to a concentration of ~1 µg/ml).
4. Incubate the membrane with the diluted antibody for 1 hr at room temperature with shaking. Alternatively, incubate at 4°C overnight.
5. Wash the membrane three times with wash buffer (for 5 min per wash).
6. Dilute a secondary antibody conjugate 1:1,000–50,000 in blocking buffer. If you are using a HRP-conjugated primary antibody, refer to the suggested dilution on the Certificate of Analysis.
7. Incubate the membrane with the diluted antibody for 1 hr at room temperature with shaking.
8. Wash the membrane three times with wash buffer (for 5 min per wash).
9. Proceed with chemiluminescent detection per the manufacturer's instructions.

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