Anti-DYKDDDDK Immunoprecipitation Protocol-At-A-Glance

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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 x 10^7–2 x 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 µl 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 µl of Wash Buffer, repeat Step 2.a, and resuspend in 30 µl 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 µl 2X SDS sample buffer. Boil samples for 5 min and spin down.
9. Load 10–15 µl 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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