Magnetic DYKDDDDK Immunoprecipitation Kit Protocol-At-A-Glance

I. Introduction

The **Magnetic DYKDDDDK Immunoprecipitation Kit** (Cat. No. 635694) may be used to purify or immunoprecipitate DYKDDDDK-tagged fusion proteins from cell lysates. The kit yields highly pure denatured proteins and is sufficient for sample preparation and immunoprecipitation of up to 50 reactions.

II. Sample Preparation and Immunoprecipitation

A. Required Materials

Supplied in Kit

- Magnetic Beads Immunoprecipitation Buffer Set (Cat. No. 635696)
    - Lysis Buffer (110 ml)
    - Wash Buffer (2 x 100 ml)
    - IP Elution Buffer (2 ml)
- Anti-DYKDDDDK Magnetic Beads (1 ml; Cat. No. 635695)
- 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.

Not Supplied

- **1X PBS**
- **Magnetic separator**—we recommend our **Magnetic Stand** (Cat. No. 631964)
- **5X SDS Sample Buffer**—see the following reference:

  Green, M. R. & J. Sambrook. (2012) Molecular Cloning: A Laboratory Manual, Fourth Edition (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

B. Protocol: Preparation of Mammalian Cell Lysate

1. Wash a 10 cm dish containing adherent cells twice with 1X PBS. Remove all residual 1X PBS. For suspension cells, transfer 1 x 10^7 cells to a 15 ml conical tube. Wash twice with 10 ml 1X PBS.
2. Add 1 ml Lysis Buffer per plate. Incubate for 15–30 min at 4°C on a rocking platform.
3. Collect cell lysate in a 1.5 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 the supernatant, which will be used in Section II.C, Step 2.

C. Protocol: Immunoprecipitation

1. Transfer 20 µl of Anti-DYKDDDDK Magnetic Beads to a separate microcentrifuge tube and wash as follows:
    a. Add 1 ml Lysis Buffer to the beads and vortex gently.
    b. Spin down briefly, then place the microcentrifuge tube on a magnetic separator (see Section II.A) and allow the magnet to pull the magnetic beads to the wall of the tube. Remove and discard the supernatant.
    c. Repeat if necessary.
2. Add 1 ml of cleared lysate from Section II.B, Step 5 to the bead pellet from Step 1. Be careful not to transfer any precipitated material as this can cause nonspecific background. If necessary, reclarify the lysate by centrifugation before adding it to the beads.
3. Incubate for 1 hr to overnight at 4°C on a rotating apparatus.
4. Collect the immunoprecipitates by placing the microcentrifuge tube on a magnetic separator and allowing the magnet to pull the magnetic beads to the wall of the tube.
5. Remove as much of the supernatant as possible without disturbing the bead pellet.
6. Wash the immunoprecipitates 3 times with 1 ml of Wash Buffer as follows:
    a. Add 1 ml Wash Buffer to the bead pellet and vortex gently.
    b. Spin down briefly, then place the microcentrifuge tube on a magnetic separator and allow the magnet to pull the magnetic beads to the wall of the tube. Remove and discard the supernatant.
    c. Repeat two more times for a total of three washes.
    d. After discarding the supernatant from the final wash, elute the beads using one of the two elution protocols described in Step 7.

    NOTE: If background is observed when purified protein samples are analyzed via Western blotting (see Section III), the wash buffer can be supplemented with higher salt concentrations (e.g., up to 1 M NaCl) or detergents such as Tween-20 or Triton X-100. Acceptable concentrations will need to be determined empirically.

7. Elute the immunoprecipitates from the beads using one of the following two elution protocols:

   - **Low pH elution protocol with Elution Buffer**

     a. Resuspend the bead pellet from Step 6.d in 20 µl of Elution Buffer (supplied in kit). Vortex gently, spin down briefly, and allow to stand for 5 min.

     b. Place the microcentrifuge tube on a magnetic separator and allow the magnet to pull the magnetic beads to the wall of the tube. Remove the supernatant and transfer it to a clean microfuge tube.

     c. Add 7 µl of 5X SDS Sample Buffer (see Section II.A) to the supernatant, which should cause it to turn blue. If not, add 1N NaOH in 1 µl increments until it does turn blue. Then proceed to Step 8.

     d. Boil the sample for 5 min and spin down briefly. Then proceed to Step 8.

   - **Alternative elution protocol with 1X SDS Sample Buffer**

     a. As an alternate to low pH elution with Elution Buffer, resuspend the bead pellet from Step 6.d in 20 µl of 1X SDS Sample Buffer (diluted from the 5X stock in Section II.A). Vortex gently, spin down briefly, and allow to stand for 5 min.

     b. Boil the sample for 5 min and spin down briefly.

     c. Place the microcentrifuge tube on a magnetic separator and allow the magnet to pull the magnetic beads to the wall of the tube. Remove the supernatant and transfer it to a clean microfuge tube. Then proceed to Step 8.

8. Load 10–15 µl of supernatant on an SDS/polyacrylamide gel. Continue with Western blotting as described in Section III.

III. Western Blotting

A. Materials Required

- 1X PBS (Alternatively, you may substitute Tris-based buffers.)
- 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

* The wash buffer used for Western blotting is not the same wash buffer supplied in the Magnetic DYKDDDDK Immunoprecipitation Kit.

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 II.C, Step 9) 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 an 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.

Contact Us For Assistance	
Customer Service/Ordering	**Technical Support**
Telephone: 800.662.2566 (toll-free)	Telephone: 800.662.2566 (toll-free)
Fax: 800.424.1350 (toll-free)	Fax: 800.424.1350 (toll-free)
Web: www.clontech.com	Web: www.clontech.com