Store at 4°C

Protein G Magnetic Beads

#70024

1mL (1 x 1mL)
5mL (5 x 1mL)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: Protein G Magnetic Beads are an affinity matrix for the small-scale isolation of immunocomplexes from immunoprecipitations (IP assays). Protein G is covalently coupled to a magnetic particle.

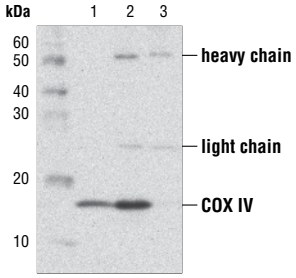
Protein G exhibits high affinity for subclasses of IgG from many species (including human, rabbit, mouse, rat, and sheep) and can be used for immunoprecipitation assays with these antibodies. Beads can be separated from solution using our 6-Tube Magnetic Separation Rack #7017 or 12-Tube Magnetic Separation Rack #14654 which concentrates the beads to the side of the tube instead of the bottom. This eliminates centrifugation steps, minimizes sample loss, increases washing efficiency, and saves time.

The 1mL and 5mL size is enough material for 25 and 125 immunoprecipitations, respectively, when following our recommended protocol.

Product Specifications:

Bead Diameter: ~1.5 µm

Binding Capacity: > 0.2 µg Rabbit IgG/µl bead slurry



Immunoprecipitation of COX IV from HeLa cells using COX IV (4D11-B3-E8) Mouse mAb #11967 and Protein G Magnetic Beads. Western blot analysis was performed on the 10% input control (lane 1), IP pellet (lane 2) and Mouse (G3A1) mAb IgG1 Isotype Control #5415 (at matched concentration) (lane 3) using COX IV (4D11-B3-E8) Mouse mAb #11967.

Storage: Supplied in PBS, 0.1% BSA, 0.004% EDTA and 0.075% sodium azide. Store at 4°C. This product is stable for 12 months.

Do not freeze, dry or centrifuge beads. This may cause irreversible aggregation and decreased binding capacity.

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Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity: H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#70024

Immunoprecipitation for Native Proteins

This protocol is intended for immunoprecipitation of native proteins for analysis by western immunoblot or kinase activity utilizing magnetic separation.

A Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L of 1X PBS, add 50 ml 20X PBS to 950 ml
2. **10X Cell Lysis Buffer:** (#9803) To prepare 10 ml of 1X cell lysis buffer, add 1 ml cell lysis buffer to 9 ml dH 2O, mix.

NOTE: Add 1 mM PMSF (#8553) immediately prior to use.

3. **3X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer.
4. **Protein G Magnetic Beads:** (#70024).
5. **Magnetic Separation Rack:** (#7017) or (#14654).
6. **10X Kinase Buffer (for kinase assays):** (#9802) To Prepare 1 ml of 1X kinase buffer, add 100 µl 10X kinase buffer to 900 µl dH 2O, mix.
7. **ATP (10 mM) (for kinase assays):** (#9804) To prepare 0.5 ml of ATP (200 µM), add 10 µl ATP (10 mM) to 490 µl 1X kinase buffer.

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate on ice for 5 min
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate on ice three times for 5 sec each.
6. Microcentrifuge for 10 min at 4°C, 14,000 x g and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at -80°C.

C Immunoprecipitation

Cell Lysate Pre-Clearing (Highly Recommended)

A cell lysate pre-clearing step is highly recommended to reduce non-specific protein binding to the Protein G Magnetic beads. Pre-clear enough lysate for test samples and isotype controls.

1. Briefly vortex the stock tube to resuspend the magnetic beads.

IMPORTANT: Pre-wash #70024 magnetic beads just prior to use:

2. Transfer 20 µl of bead slurry to a clean tube. Place the tube in a magnetic separation rack for 10-15 seconds. Carefully remove the buffer once the solution is clear. Add 500 µl of 1X cell lysis buffer to the magnetic bead pellet, briefly vortex to wash the beads. Place tube back in magnetic separation rack. Remove buffer once solution is clear. Repeat washing step once more.
3. Add 200 µl cell lysate to 20 µl of pre-washed magnetic beads.

IMPORTANT: The optimal lysate concentration will depend on the expression level of the protein of interest. A starting concentration between 250 µg/ml-1.0 mg/ml is recommended.

4. Incubate with rotation for 20 minutes at room temperature.
5. Separate the beads from the lysate using a magnetic separation rack, transfer the pre-cleared lysate to a clean tube, and discard the magnetic bead pellet.
6. Proceed to immunoprecipitation section.

Immunoprecipitation

IMPORTANT: Appropriate isotype controls are highly recommended in order to show specific binding in your primary antibody immunoprecipitation. Use Normal Rabbit IgG #2729 for rabbit polyclonal primary antibodies, Rabbit (DA1E) mAb IgG XP ® Isotype Control #3900 for rabbit monoclonal primary antibodies, and Mouse (G3A1) mAb IgG1 Isotype Control #5415 for mouse monoclonal primary antibodies. Isotype controls should be concentration matched and run alongside the primary antibody samples.

1. Add primary antibody (at the appropriate dilution as recommended in the product datasheet) to 200 µl cell lysate. Incubate with rotation overnight at 4°C to form the immunocomplex.
2. Pre-wash magnetic beads (see Cell Lysate Pre-Clearing section, steps 1 and 2).
3. Transfer the lysate and antibody (immunocomplex) solution to the tube containing the pre-washed magnetic bead pellet.
4. Incubate with rotation for 20 min at room temperature.
5. Pellet beads using magnetic separation rack. Wash pellets five times with 500 µl of 1X cell lysis buffer. Keep on ice between washes.
6. Proceed to analyze by western immunoblotting or kinase activity (section D).

D Sample Analysis

Proceed to one of the following specific set of steps.

For Analysis by Western Immunoblotting

1. Resuspend the pellet with 20-40 µl 3X SDS sample buffer, briefly vortex to mix, and briefly microcentrifuge to pellet the sample.
2. Heat the sample to 95–100°C for 5 min
3. Pellet beads using magnetic separation rack. Transfer the supernatant to a new tube. The supernatant is the sample.
4. Load the sample (15–30 µl) on SDS-PAGE gel.
5. Analyze sample by western blot (see Western Immunoblotting Protocol).

NOTE: To minimize masking caused by denatured IgG heavy chains (~50 kDa), we recommend using Mouse Anti-Rabbit IgG (Light-Chain Specific) (D4W3E) mAb (#45262) or Mouse Anti-Rabbit IgG (Conformation Specific) (L27A9) mAb (#3678) (or HRP conjugate #5127). To minimize masking caused by denatured IgG light chains (~25 kDa), we recommend using Mouse Anti-Rabbit IgG (Conformation Specific) (L27A9) mAb (#3678) (or HRP conjugate #5127).

For Analysis by Kinase Assay

1. Wash pellet twice with 500 µl 1X kinase buffer. Keep on ice.
2. Suspend pellet in 40 µl 1X kinase buffer supplemented with 200 µM ATP and appropriate substrate.
3. Incubate for 30 min at 30°C.
4. Terminate reaction with 20 µl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 sec.
5. Transfer supernatant containing phosphorylated substrate to another tube.
6. Heat the sample to 95–100°C for 2–5 min and microcentrifuge for 1 min at 14,000 x g.
7. Load the sample (15–30 µl) on SDS-PAGE gel.

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