Absolute Mag™ Anti-Goat IgG Magnetic Particles

Immunoprecipitation Protocol

Description

Absolute Mag™ Anti-Goat IgG Magnetic Particles are ideal for antibody purification and immunoprecipitation assays. Absolute Mag™ Anti-Goat IgG recognize and efficiently bind to the Fc part of immunoglobulin (Ig) following a short incubation. The generated particle-antibody complex can be separated from the rest of the sample by magnet. The retained antibody can be eluted from the particles using an elution buffer.

Absolute Mag™ particles enable identification of new protein-protein interactions through immunoprecipitation assays, where the Absolute Mag™ anti-goat IgG -antibody complex can be used to isolate particular proteins of interest or protein complex from assay samples, e.g. cell lysate. The immunoprecipitated proteins can be further analyzed by electrophoresis, protein staining, and mass spectrometry. Absolute Mag™ particles are much smaller than conventional micro-beads. This feature allows for better accessibility of the particles to the antigenic epitope and for less disturbance to the native functions of proteins or protein-protein complexes. In addition, the surfaces of Absolute Mag™ particles are uniquely coated to reduce non-specific interactions with cellular proteins and other biomolecules. This feature allows for a more specific 'pull down' of real protein complex targets.

Product Contents

Absolute Mag™ anti-goat IgG particles are provided in phosphate buffered saline (PBS), pH 7.4. Each vial contains 1 ml of solution, which is enough for approximately 40 ug antibody enrichment.

All materials should be stored at 4°C up to 8 months.

Immunoprecipitation Protocol

1. Add 1 ug of antibody (or recommended amount following company protocol) to the tube containing precleared lysate.
2. Incubate for an hour with mixing at 4° C.
3. Add 25ul of CD Anti-Goat IgG particles to the tube. Rotate for 1-2 hours at 4°C.
4. Separate the particles from sample solution (cell lysate) with magnet. Remove supernatant.
5. Wash the particles 3 times with 50ul of lysis buffer used.
6. After the last wash, remove the supernatant and add elution buffer to the beads.

Elute antibody and proteins by using either the denaturing elution methods or the non-denaturing elution method.

A. Denaturing elution:

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1) Add 20-30 ul of SDS-PAGE protein sample buffer to the particle-antibody-protein complex, gently pipette, and boil the sample in water bath for 5 minutes.
2) Place the tube on the magnet to separate the particles, and load the supernatant onto a gel.

B. Non-denaturing elution

1) Add 20-30 ul of Elution Buffer to the particle-antibody-protein complex, gently pipette, and incubate for 1-2 minutes.
2) Place the tube on the magnet to separate the particles, and transfer the supernatant to a clean tube. If neural pH is desired for further analysis, add 2-3 ul of I M Tris (pH8) to the sample.

7. Vortex and incubate for 5 minutes.
8. Magnetically separate particles from the solution. Collect supernatant while avoiding disturbing the bead pellet. The target proteins are in the supernatant and ready to be analyzed.

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