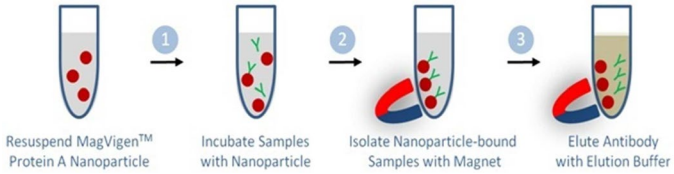
MagVigen™ - anti-Mouse Antibody

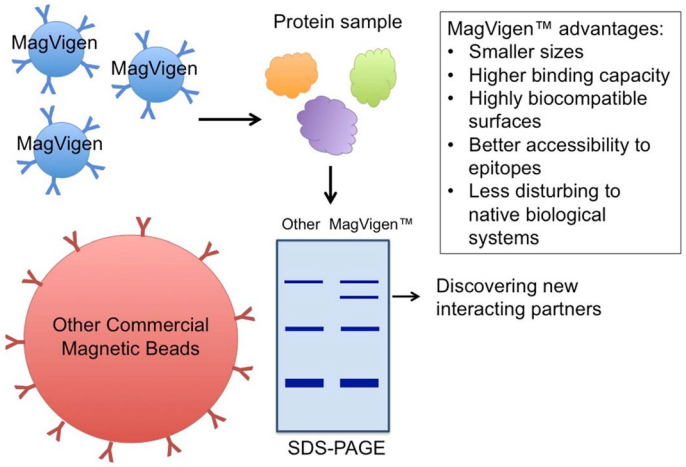
Cat # 21007/K21007

Product Description

MagVigen™-anti-Mouse Antibody are ideal for purification of mouse antibody tagged proteins or cells in immunoprecipitation assays or cellular assays. The subsequent nanoparticle bound proteins, protein complexes or cells can be easily separated from the rest of the sample by a magnet. The retained proteins can be eluted from the nanoparticles using an elution buffer.



MagVigen™ nanoparticles enable identification of new protein-protein interactions through immunoprecipitation assays, where the MagVigen™ - protein 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. MagVigen™ nanoparticles are much smaller than conventional micro-beads, and they have optimal surface chemistry. These features allow for better accessibility of the nanoparticles to the antigenic epitope and for less disturbance to the native functions of proteins or protein-protein complexes. In addition, the surfaces of MagVigen™ nanoparticles 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 and Related Product Contents

- Cat# 21007: MagVigen™-anti-Mouse antibody conjugates (Cat # 21006) are provided in phosphate buffered saline (PBS), pH 7.4. Each vial contains 1 ml of solution with a particle concentration of 1 mg/ml, which is enough for approximately 5-50 antibody enrichment or immunoprecipitation assays, or for binding 50 million cells.

Cat# K21007 further includes:

- Washing Buffer (10X), 15 ml, Cat# A20001.
- Elution Buffer, 15 ml, Cat# A20002.

16 sample magnetic rack, Cat# A20006 (not included).

All materials except Magnet should be stored at 4°C.

Shelf life: 6 months.

Protocol

This protocol provides guidance for protein purification, immunoprecipitation assay, and cell separation using MagVigen™ -anti-Rabbit antibody conjugates. Optimization may be needed for specific application.

I. Protein Purification

1. Dilute 10X Washing Buffer with PBS to 1X.
2. Gently vortex MagVigen™ nanoparticles before use.
3. Take 20-50 µl nanoparticle solution (for 0.5-5 µg of Mouse antiobdy) and add it to 100 µl 1X Washing Buffer, and mix well.
4. Separate the nanoparticles from the solution by placing the magnet on the side of the tube for 1-2 min and remove the supernatant carefully (with magnet still on the side). Note: A clear precipitate containing dark brown colored nanoparticles should become visible on the side of the micro-centrifuge tube.
5. Remove magnet and wash the nanoparticles with 100 µl 1X Washing Buffer. Repeat step 4, and remove supernatant.
6. Add 100 µl sample solution containing desired mouse antibody to the nanoparticle pellet, mix well, and incubate with gentle rotation for 0.5-2 hours at room temperature or 4 °C overnight.
7. After incubation, use the magnet to separate MagVigen™-Mouse antibody conjugates from the solution and remove the supernatant.
8. Wash MagVigen™-Mouse antibody conjugates with 100 µl 1X Washing Buffer twice and remove supernatant.
9. Elute captured protein from the nanoparticles by adding 90 µl Elution Buffer, mix well, and incubate for 2-5 min at room temperature.
10. Separate the nanoparticles from the eluted mouse antibody with magnet. Transfer supernatant to a clean tube and immediately neutralize the eluate by adding 10 µl Tris (1M, pH=8.0). The purified protein is ready to use for subsequent evaluation.
    Note: some Mouse antibody may not be stable using this elution method (steps 9 and 10). Please choose appropriate elution method for specific application.

II. Immunoprecipitation (from cell lysate)

Steps 1-8 are the same as Protein Purification

9. Add cell lysate sample, typically 100-1000 µl, to nanoparticle pellet and gently pipette to mix.
10. Incubate the reaction by rotating for 1-2 hours at room temperature or 4 °C to allow the antigen to bind to the MagVigen™-Mouse antibody conjugates.
    Note: depending on the affinity of protein-protein interaction, the incubation time can be adjusted for optimal binding.
11. After incubation, use the magnet to separate the nanoparticle- protein complex from the solution, and remove the supernatant.
12. Wash nanoparticle-protein complex with 100 µl of 1X Washing Buffer for three times.
13. Elute antibody and proteins by using either the denaturing elution methods or the non-denaturing elution method.

A. Denaturing elution:

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

B. Non-denaturing elution

1) Add 20-30 µl of Elution Buffer to the nanoparticle-protein complex, gently pipette, and incubate for 2-5 minutes.
2) Place the tube on the magnet to separate the nanoparticles, and transfer the supernatant to a clean tube. If neural pH is desired for further analysis, add Tris (1 M, pH=8) to the sample.

III. Cell Separation

This protocol provides a general guidance for enriching 10^5 cells using MagVigen™- anti-Mouse IgG magnetic nanoparticles. Please adjust the amount of reagents for specific application.

Steps 1-2 are the same as Protein Purification

3. Aliquot 20 µl MagVigen-anti Mouse nanoparticle solution for cell separation experiment. Note: 20 µl is generally sufficient for the enrichment of (1-10) x 10^5 cells. Cell capture efficiency can be affected by factors such as frequency of target cells in the cell population, density of antigen/epitope expressed on the cell surface, and the antibody affinity. Adjust the amount of nanoparticles accordingly.
4. Wash nanoparticles with 500 µl 1X Washing Buffer twice. Separate the nanoparticles from the solution by placing the magnet on the side of the tube for 1-2 min and remove the supernatant carefully (with magnet still on the side).
5. Add 250 ng primary Mouse antibody to the nanoparticle and incubate for 30-60 minutes using a sample rotator.
   Note: 20 µl nanoparticles could bind up to 1 µg antibody.
6. Wash nanoparticle-Mouse antibody conjugates with 500 µl 1X Washing Buffer twice to remove unbound antibody.
7. Resuspend the nanoparticle-Mouse antibody conjugates in 1X Washing Buffer (20 µl) and add it to the cell sample to a total volume of 0.1-0.5 ml.
8. Incubate the nanoparticles with the cell sample on an orbital shaker for 30 minutes at room temperature.
9. After incubation, use a magnet to separate the nanoparticles (with bound cells) from the solution, and carefully remove the supernatant.
10. Wash the nanoparticles with 500 µl cell culture medium twice.
11. Isolated cells can be re-suspended in cell culture medium for downstream applications.
    Note: Primary mouse antibody can also be directly added to cell suspension, and then apply nanoparticles for cell capturing.