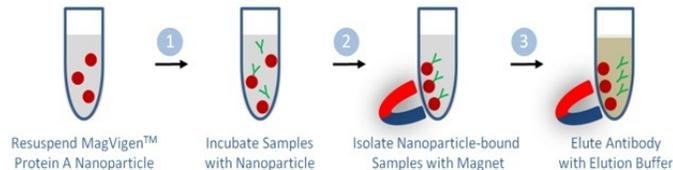


MagVigen™ - anti-rabbit IgG nanobeads/kit

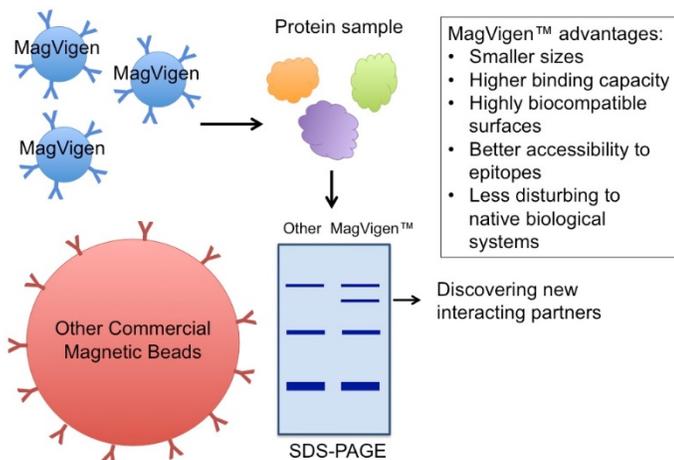
Cat # 21006/K21006

Product Description

MagVigen™ - anti-rabbit IgG nanoparticles are ideal for antibody purification and immunoprecipitation assays. MagVigen™ Protein anti-rabbit IgG recognize and efficiently bind to the Fc part of immunoglobulin (Ig) following a short incubation. The generated nanoparticle-antibody complex can be separated from the rest of the sample by magnet. The retained antibody 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™ anti-rabbit 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. MagVigen™ nanoparticles are much smaller than conventional micro-beads. This feature allows 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 Contents

- Cat# 21006: MagVigen™ - anti-rabbit IgG nanoparticles (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 20-200 antibody enrichment or immunoprecipitation assays.

Cat# K21006 further includes:

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

- Elution Buffer, 15 ml, Cat# A20002.

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

Shelf life: 6 months.

Protocol

Antibody Enrichment

This protocol was optimized for enrichment of 1-10 µg rabbit or mouse antibody in a volume of 100 µl. For a smaller size of sample, it is recommended to add extra Washing Buffer to reach a 100 µl reaction volume. For larger scale of purification, adjust the amount of reagents accordingly.

1. Dilute 10X Washing Buffer with PBS to 1X.
2. Vortex MagVigen™ nanoparticles for 10-20 seconds.
3. Take 5-50 µl nanoparticle solution (for 1-10 µg antibody), add it to 100 µl 1X Washing Buffer, and vortex to mix.
4. Separate the nanoparticles from the solution by placing the magnet on the side of the tube for 2-5 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 antibodies to the nanoparticle pellet, mix well, and incubate with gentle rotation for 2 hours at room temperature or 4 °C overnight.
7. After incubation, use the magnet to separate nanoparticle-antibody complex from the solution and remove the supernatant.
8. Wash nanoparticle-antibody complex with 100 µl 1X Washing Buffer twice and remove supernatant.
9. Elute captured antibody from the nanoparticles by adding 90 µl Elution Buffer, mix well, and incubate for 1 min at room temperature.
10. Separate the nanoparticles from the eluted antibody with magnet. Transfer supernatant to a clean tube and immediately neutralize the eluate by adding 10 µl Tris (1M, pH=8.0). The enriched antibody is ready to use for subsequent evaluation.

Immunoprecipitation (from cell lysate)

Steps 1-8 are the same as **Antibody Enrichment**

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™-antibody complex.
Note: depending on the affinity of the antibody used, the incubation time can be adjusted for optimal binding.
11. After incubation, use the magnet to separate the nanoparticle-antibody-protein complex from the solution, and remove the supernatant.
12. Wash nanoparticle-antibody-protein complex with 100 µl of 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-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 nanoparticles, and load the supernatant onto a gel.

B. Non-denaturing elution

- 1) Add 20-30 µl of Elution Buffer to the nanoparticle-antibody-

protein complex, gently pipette, and incubate for 1-2 minutes.

2) Place the tube on the magnet to separate the nanoparticles, and transfer the supernatant to a clean tube. If neutral pH is desired for further analysis, add Tris (1 M, pH=8) to the sample.

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

Protein Purification

1. Dilute 10X Washing Buffer with PBS to 1X.
2. Gently vortex MagVigen™ nanoparticles before use.
3. Take 20-50 μ l nanoparticle solution (for 0.5-5 μ g of Rabbit antibody) and add it to 100 μ l 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 μ l 1X Washing Buffer. Repeat step 4, and remove supernatant.
6. Add 100 μ l sample solution containing desired rabbit 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™-Rabbit antibody conjugates from the solution and remove the supernatant.
8. Wash MagVigen™-Rabbit antibody conjugates with 100 μ l 1X Washing Buffer twice and remove supernatant.
9. Elute captured protein from the nanoparticles by adding 90 μ l Elution Buffer, mix well, and incubate for 1 min at room temperature.
10. Separate the nanoparticles from the eluted rabbit antibody with magnet. Transfer supernatant to a clean tube and immediately neutralize the eluate by adding 10 μ l Tris (1M, pH=8.0). The purified protein is ready to use for subsequent evaluation.
Note: *some Rabbit antibody may not be stable using this elution method (steps 9 and 10). Please choose appropriate elution method for specific application.*

Cell Separation

1. Dilute 10X Washing Buffer with PBS to 1X.
2. Gently vortex MagVigen™ nanoparticles before use.
3. Aliquot 20 μ l MagVigen-anti Rabbit nanoparticle solution for cell separation experiment. Note: 20 μ l is generally sufficient for the enrichment of (1-10) $\times 10^5$ 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 μ l 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 Rabbit antibody to the nanoparticle and incubate for 30-60 minutes using a sample rotator.
Note: *20 μ l nanoparticles could bind up to 1 μ g antibody.*
6. Wash nanoparticle-Rabbit antibody conjugates with 500 μ l 1X Washing Buffer twice to remove unbound antibody.
7. Resuspend the nanoparticle-Rabbit antibody conjugates in 1X Washing Buffer (20 μ l) 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 μ l cell culture medium twice.
11. Isolated cells can be re-suspended in cell culture medium for downstream applications.
Note: *Primary rabbit antibody can also be directly added to cell suspension, and then apply nanoparticles for cell capturing.*