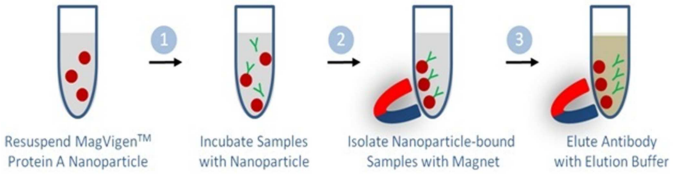
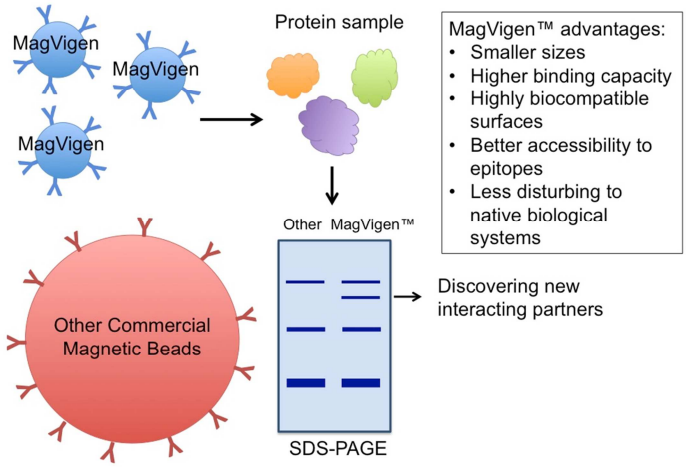
MagVigen™ - anti-FLAG Nanoparticles

Product Description

MagVigen™-anti-FLAG nanoparticles are ideal for the purification of FLAG tagged recombinant proteins and immunoprecipitation assays. The subsequent nanoparticle-FLAG protein complex can be easily separated from the rest of the sample by magnet. The retained FLAG fusion 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™ -FLAG 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. 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

- MagVigen™-anti-FLAG nanoparticles 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 20-50 antibody enrichment or immunoprecipitation assays.
- Washing Buffer (10X), 15 ml
- Elution Buffer, 15 ml
- Magnet

All materials except Magnet should be stored at 4°C up to 6 months.

Protocol

Protein purification

This protocol provides guidance for protein purification using MagVigen™ -anti-FLAG nanoparticles. Optimization may be needed for specific application.

1. Dilute 10X Washing Buffer with PBS to 1X.
2. Mix well MagVigen™ nanoparticles before use.
3. Take 20-50 µl nanoparticle solution (for 0.1-1 µg FLAG fusion protein), add it to 100 µl 1X Washing Buffer, and mix.
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 FLAG fusion protein 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 nanoparticle-protein complex from the solution and remove the supernatant.
8. Wash nanoparticle-protein complex 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 1 min at room temperature.
10. Separate the nanoparticles from the eluted FLAG fusion protein 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 FLAG fusion proteins may not be stable using this elution method (steps 9 and 10). Please choose appropriate elution method for specific application.

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™-FLAG protein complex. **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 1-2 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.