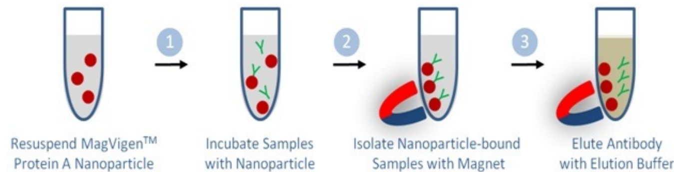
MagVigen™ - anti-FLAG, mouse Nanobeads/Kit,

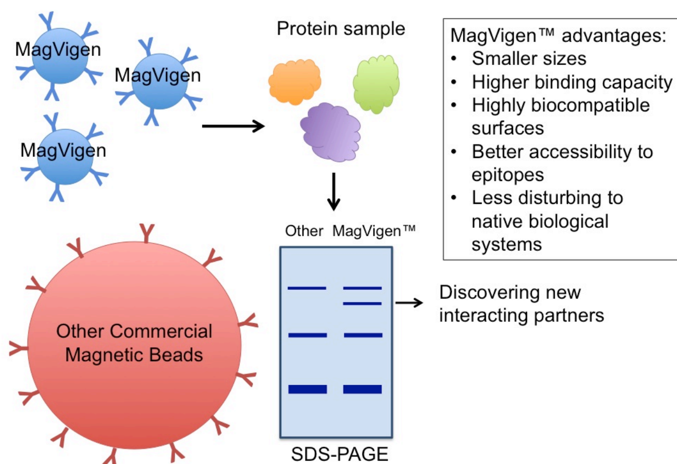
Catalog # 51007/K51007 v.1810101

Product Description

MagVigen™ - Anti-FLAG nanoparticles are ideal for FLAG-tagged protein purification and immunoprecipitation assays. MagVigen™ Anti-FLAG recognizes and efficiently binds to FLAG-tagged molecules following a short incubation. The generated nanoparticle-FLAG-tag molecule 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-FLAG – FLAG tagged molecule 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 (Cat # 51007) 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# K51007 only: Washing Buffer (10X), 15 ml

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

Protocol

Antibody Enrichment

This protocol was optimized for enrichment of 1-10 µg 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 (customer choice), 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 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-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 neural pH is desired for further analysis, add Tris (1 M, pH=8) to the sample.