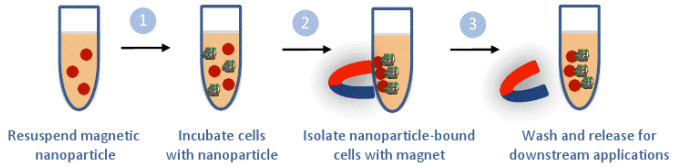
MagVigen™ Streptavidin conjugates/kit

Cat# 21005C/K21005C

Product Description

MagVigen™-Streptavidin magnetic nanoparticles can universally bind to any biotinylated biomolecules (ex. antibody, protein, peptide, DNA) through high affinity interaction between streptavidin and biotin. The MagVigen™-Streptavidin-biotin-biomolecule complex can be easily separated from unbound biotin-biomolecule using a magnetic rack (Cat#A20006). This provides a quick and neat way to tag biomolecules with magnetic nanoparticles. The purified nanoparticle-biomolecule complex can be used in a variety of downstream bio-separation processes (ex. protein purification, immunoprecipitation, cell isolation or depletion, and molecular detection.)

MagVigen™- Streptavidin are ideally used together with mouse antibody for isolation of cells (e.g. CTCs, stem cells) from a mixture of cell population obtained from tissues or organs. The isolated cells are viable and can be further cultured or used for downstream molecular analysis such as mRNA isolation and RT-PCR. Cell separation with MagVigen™ nanoparticles eliminates the use of columns, so cells are not exposed to the mechanical stress from passing through the column matrix. Magnetically separated cells are highly purified and retain their viability, ideal for downstream applications.



Advantages of MagVigen™ - Streptavidin for cell enrichment

- Easy and quick to make nanoparticle-primary mouse antibody conjugates
- Simple and gentle cell separation
- Strong and long-lasting fluorescent signal
- Consistent, high quality results
- High binding capacity
- High biocompatibility
- Low non-specific binding

Product Contents

- Cat# 21005C: MagVigen™- Streptavidin (Cat# 21005) are provided in phosphate buffered saline (PBS) containing 0.02% NaN3, 0.02% BSA. pH 7.4. Each vial contains 1 ml of solution with particle concentration of 2 mg/ml, which is enough for binding 10^9 cells.

  Nanoparticle size: 200-500 nm measured using Dynamic Light Scattering.

  Polydispersity index < 0.2.

  Capacity: 50µg biotin-antibody/ml of nanoparticles

Cat# K21005C further includes:

- Washing Buffer
- Elution Buffer

All materials except the magnet should be stored at 4°C for up to 6 months.

Protocol

Cell Enrichment

This protocol provides a general guidance for enriching 10^5 -10^6 cells using MagVigen™- Streptavidin magnetic nanoparticles. Please adjust the amount of reagents for specific application.

1. Gently vortex or pipette the MagVigen™- Streptavidin magnetic nanoparticles in the vial before use.
2. Aliquot 20-50 µl nanoparticle solution for enrichment experiment.

   Note: *20-50 µl is generally sufficient for the enrichment of 10^5 - 10^6 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.*
3. Wash nanoparticles with 500 µl of 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).
4. Add 1µg-2µg biotin-conjugated antibody (in a volume of 100-200 µl) to the nanoparticle and incubate for 30-60 minutes on a rotator.

   Note: *50 µl nanoparticles could bind ~2µg of antibody.*
5. Wash nanoparticle-antibody conjugates with 500 µl Washing Buffer twice to remove unbound antibody.
6. Resuspend the nanoparticle-antibody conjugates in Washing Buffer (50 µl) and add it to the cell sample to a total volume of 0.1-0.5 ml.
7. Incubate the nanoparticles with the cell sample on an orbital shaker for 30 minutes at room temperature.
8. After incubation, use a magnet to separate the nanoparticles (with bound cells) from the solution, and carefully remove the supernatant.
9. Wash the nanoparticle-cell complex with 500µl cell culture medium twice.
10. Isolated cells can be re-suspended in cell culture medium for downstream applications.

Note: *Elution buffer can be used to elute the target protein or cells from MagVigen™-Streptavidin magnetic nanoparticles.*

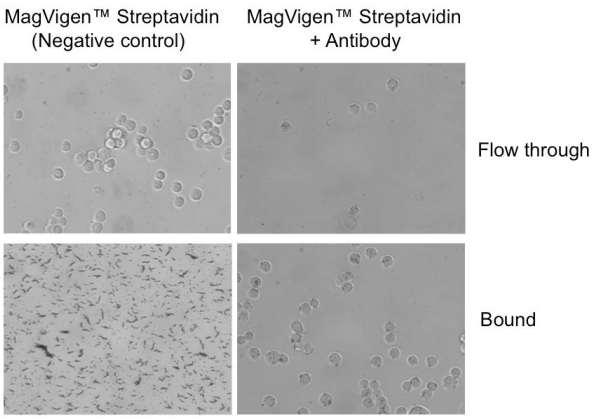
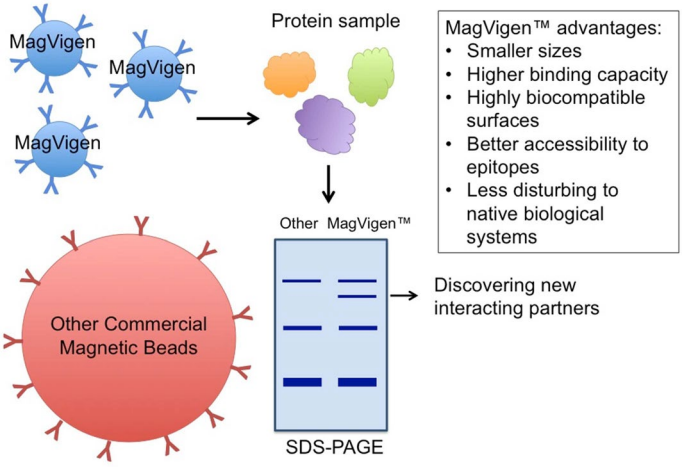


Figure 1. Enrichment of tumor cells by MagVigen™ Streptavidin-antibody nanoparticles. Representative images from experiment starting from 5x10^5 cells are shown.

Immunoprecipitation

MagVigen™ nanoparticles enable identification of new protein-protein interactions through immunoprecipitation assays, where the MagVigen™ streptavidin – biotin-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.



Nanoparticle Wash

For optimal results from the nanoparticles, it is recommended that the nanoparticles are washed prior to addition to samples.

1. Vortex MagVigen™ nanoparticles for 10-20 seconds.
2. Take 50µl of nanoparticle solution, add it to 100µl 1X Washing Buffer, and vortex to mix.
3. 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.*
4. Wash nanoparticles 2 times.

Immunoprecipitation

5. Add 2 µg of biotin-antibody (or recommended amount following company protocol) to the tube containing cell lysate.
6. Incubate on ice for an hour.
7. Add 50µl of pre-washed MagVigen™ nanoparticles to the tube. Rotate for 2 hours at 4°C.
8. Separate the nanoparticles from sample solution (cell lysate) with magnet. Remove supernatant.
9. Wash the nanoparticles 3 times with 50µl of lysis buffer used.
10. After the last wash, remove the supernatant and add 100µl of elution buffer to the beads.
11. Vortex and incubate for 5 minutes.
12. Magnetically separate nanoparticles from the solution. Collect supernatant while avoiding disturbing the bead pellet. The target proteins are in the supernatant and ready to be analyzed.

Exosome isolation

1. Gently vortex or pipette the MagVigen™- Streptavidin magnetic nanoparticles in the vial before use.
2. Aliquot 50 µl nanoparticle solution for enrichment experiment. ***Note:*** *50 µl is generally sufficient for the enrichment of 1-10x10^5 exosomes. Exosome capture efficiency can be affected by factors such as frequency of exosomes in the sample, density of antigen/epitope expressed on the exsosome surface, and the antibody affinity. Adjust the amount of nanoparticles accordingly.*
3. Wash nanoparticles with 500 µl of 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).
4. Add 500 ng biotin-conjugated antibody (in a volume of 100-200 µl) to the nanoparticle and incubate for 30-60 minutes on a rotator.

   Note: *50 µl nanoparticles could bind ~200 ng of antibody.*
5. Wash nanoparticle-antibody conjugates with 500 µl Washing Buffer twice to remove unbound antibody.
6. Resuspend the nanoparticle-antibody conjugates in Washing Buffer (50 µl) and add it to the cell sample to a total volume of 0.1-0.5 ml.
7. Incubate the nanoparticles with pre-enriched exosome sample on an orbital shaker for 2 hours or overnight at 2°C – 8°C.
8. After incubation, use a magnet to separate the nanoparticles (with bound exosomes) from the solution, and carefully remove the supernatant.
9. Wash the nanoparticle-exosome complex with 500µl Washing Buffer.
10. The exosome bound nanoparticles are now ready for exosome lysis, gel electrophoresis, and western analysis.