MagVigen™ - SH Surface

Cat # 21002

Product Description

MagVigen™ - SH surface nanoparticles provide you with the flexibility of coupling to various molecules through simple bioconjugation reactions. The resulting MagVigen™ bioconjugates could be exploited to achieve highly specific binding for cell isolation, protein, DNA/RNA purification or immunoprecipitation assays. Examples of biomolecules that could be covalently bound to MagVigen™ surfaces include primary antibody, protein/peptide, DNA/RNA or other ligands.

Advantages of MagVigen™ magnetic nanoparticles

- Magnetically responsive to a magnet, easy for bio-conjugation and purification
- Smaller nanoparticle size, higher binding capacity, longer settling time, compatible to automation and high throughput workflow
- Optimal surface chemistry, low non-specific binding
- Consistent, high quality results

Product and Related Product Contents

- MagVigen™ - SH surface nanoparticles (Cat# 21002) are provided in phosphate buffered saline (PBS), pH 7.4. Each vial contains 1 ml of solution with a particle concentration of 4 mg/ml.
- Washing Buffer (10X), Cat# A20001.
- Magnet, Cat# A20003.

All materials except the magnet should be stored at 4°C for up to 1 year.

Protocol

Antibody Conjugation to MagVigen™ – SH Surface

1. Determine needed surface coverage of antibody per nanoparticle.
   Note: *The general range is about 0.3 -1.5 mg antibody per mg of MagVigen™.*
   Mix Antibody in water with SMCC (Succinimidyl trans-4(maleimidylmethyl) cyclohexane-1-carboxylate) based crosslinker in PBS solution. Incubate for 40-60 min.
   Note: *SMCC is used to crosslink the amine groups on antibody with the –SH groups from MagVigen™. SMCC based crosslinker is suggested because of its superior chemical stability when used with our nanoparticles and its ease of use.*
   Note: *The ratio of SMCC to antibody is 1:10.*
2. Desalt Antibody-SMCC using NAP column.
   Note: *This step removes the free SMCC from the Antibody-SMCC mixture.*
3. Mix purified Antibody-SMCC with MagVigen™-SH nanoparticles. Incubate overnight under continuous rotation at room temperature.
4. Separate out MagVigen™-Antibody by magnetic purification.
5. Wash 1-3 times with PBS or other buffer solution. Remove non-magnetically captured solution.
   Note: *One wash could be sufficient for most applications.*

Resuspend washed MagVigen™-Antibody conjugates into preferred buffer, ready to use.

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.

MagVigen™ for Antibody Purification and Concentrating:
General Optimal Proportion: 1 mg of magnetic beads for 50 µg of antibody. The proportion may be optimized based on specific antibody or protein property.

Note for Large Volume (>50 ml) Protein Purification:

- **Increase incubation time to ensure yield**
- **Increase magnetic pull down time to ensure majority of beads forming the pellet, this could be confirmed by total clearness of the supernatant.**