SPHERO™ Technical Note **STN-22 Rev A 050520**

SPHERO™ ANTIBODY COATED PARTICLES USES AND PROTOCOLS

INTRODUCTION

Since the 1950's, latex based immunoassays have been used in clinical laboratories. The first described latex agglutination assay was developed by Plotz and Singer as a Rheumatoid Factor test in 1956. Currently, in the life sciences immunoassays are used for detecting different proteins, hormones, and antibodies. In industry, immunoassays are used to detect pathogens in food and water and for monitoring specific molecules in pharmaceuticals and drug discovery. Four types of bead based assays are regularly used in these applications; Elisa type sandwich assays, antigen based sandwich assays, competitive inhibition assay, and rapid screening assays. The use of secondary antibody coated beads improves the development of immunoassys since monoclonal IgG attached to the SPHERO™ Antibody Coated Beads bind to target analyte effeciently.

Immunoassay using SPHERO™ Antibody Coated Beads

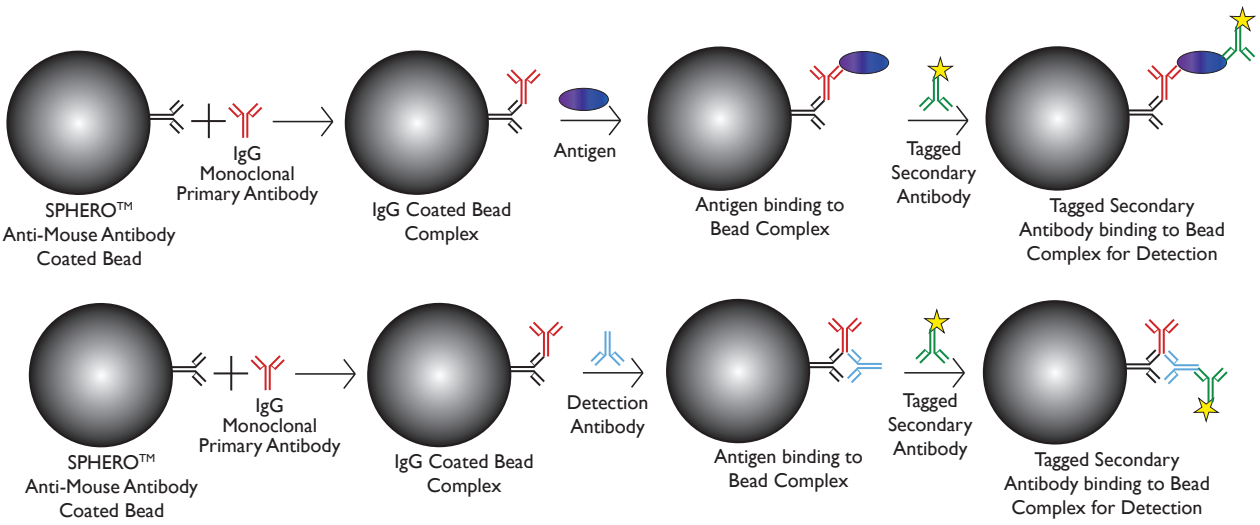
In a typical bead based sandwich immunoassay, a monoclonal antibody is covalently coupled onto a functionalized beads. In some instances, the covalent coupling of the monoclonal to fuctionalized beads has limitations and is not efficient in target analyte detection. After the covalent coupling of the monoclonal antibody, there can be a decrease in its binidng affinity because of unoptimal site-specific immobilization and orientation. In order to eliminate these issues, SPHERO™ Antibody Coated Beads beads are used to immobilize primary antibodies, the capture antibody, to improve target antigen and antibody detection. The primary antibodies bind to the heavy chains of anti-mouse antibodies on the bead's surface leaving the By using a secondary antibody coated beads, any interaction between the binding sites of the primary antibody and the reactive groups on the functionalized bead is eliminated. The secondary antibodies bind to the heavy chains of primary antibodies leaving the primary antibody's Fab domain available to bind to an antigen.

As a result, when the test sample contining the analyte of interest is added to the primary antibody coated beads, the primary antibody will bind the target antigen effectively improving the detection sensitivity. Likewise, SPHERO™ Antibody Coated are used in assay development during competitive inhibition and rapid assays. In these assays, the secondary antibody also orientates the monoclonal antibodies for effective antigen binding.

Protocol for developing a bead based assay:

1. Monoclonal antibodies are added and bound to the SPHERO™ Antibody Coated bead in an appropriate coupling buffer such as 1x PBS, 7.4, incubated for 30 minutes at room temperature, and washed with 1x PBS, 7.4 to remove any unbound monoclonal antibody.
2. A biological sample such as serum, urine or plasma is added to the primary and secondary antibodies bead complex from step 1. The target antigen or antibody binds to the monoclonal antibody of the bead complex. The beads are then washed with 1x PBS, 7.4 to remove any unbound analyte from the beads.
3. An enzyme or fluorophore conjugated antibody, either monoclonal or polyclonal, is added to the target analyte bound bead complex for target detection. The beads are then washed with 1x PBS, 7.4 to remove unbound enzyme or fluorophore conjugated antibody.
4. If an enzyme-conjugated antibody is used, a colorimetric substrate is added for detection. The colorimetric substrate will generate a color signal proportional to the amount of target antigen present in the original sample added.

Depending on the immunoassay format, the degree of signal detection can be detected or measured with the naked eye, a scintillation counter, a spectrophotometric plate reader, or with a flow cytometer.



Development of Immunoassays using SPHERO™ anti-Mouse Antibody Coated Beads

INSTRUCTIONS FOR USE

Low protein binding microcentrifuge plastic tubes are recomended while using SPHERO™ Antibody Coated Particles. The beads should be washed and resuspended in 1x PBS (pH 7.4) to improve binding. The pH in the sample containing IgG monoclonal antibody might be adjusted for the same reason. If the monoclonal IgG antibody contains BSA, sodium azide , or other materials, filter using a Thermo Scientific™ Zeba™ Spin Desalting Column before adding to SPHERO™ Antibody Coated Particles for a better coating.

The following procedure described is for the isolation of Mouse IgG from a 100 µl sample from 2 µg/ml Mouse IgG using Spherotech catalog number MPFc-60-5 (Goat anti-Mouse IgG (Fc) Coated Polystyrene Particles, 6.0-8.0 µm, 0.5% w/v, 5mL). The concentration of the beads should be adjusted according to the concentration of the analyte of interest in the sample. However, please reference the technical product insert for the binding capacity of the beads to be coated. The coating procedure involves two steps:

1. Washing the anti-Mouse beads
2. Coating the anti-Mouse beads with Mouse IgG

Goat anti-Mouse IgG (Fc) Coated Beads Wash Procedure

1. Resuspend the MPFc-60-5 beads thoroughly, in the vial (e.g. by light vortexing 1-2 minutes, pipetting up and down for 1-2 minutes, or rotating on a roller).
2. Transfer 15-100µl of the MPFc-60-5 bead solution to a microcentrifuge tube at room temperature.
3. Place the test tube in a centrifuge at 1,500x *g* for 20 minutes and remove the supernatant using a pipette.
4. Add 0.5mL of 1x PBS, pH 7.4
5. Repeat steps 3, 4 and 3 again.

Mouse IgG Coating Procedure

1. Add Mouse IgG dissolved in 1x PBS, pH 7.4 to the washed beads. Normally, 10µg of Mouse IgG is good starting point for 0.5% w/v, 5mL of MPFc-60-5 beads. The binding of the Mouse IgG is dependent on the purity of the antibody and binding efficiency. 2. Incubate using a microcetrifuge tube bench shaker with gentle mixing for 30 minutes at room temperature. 3. Centrifuge at 1,500x g for 20 minutes and remove the supernatant. 4. Wash beads 2 times with 1x PBS, pH 7.4 to remove excess Mouse IgG from the bead solution. 5. For downstream immunoprecipitation or storage, 0.01-0.1% Tween-20 can be added to the buffer to prevent aggregation of the Ig-coupled beads. The Mouse IgG coated beads are now ready for immunoassay development using an antigen or antibody analyte for detection.

Sandwich Assay Using Goat anti-Mouse IgG (Fc) Coated Beads

Materials:

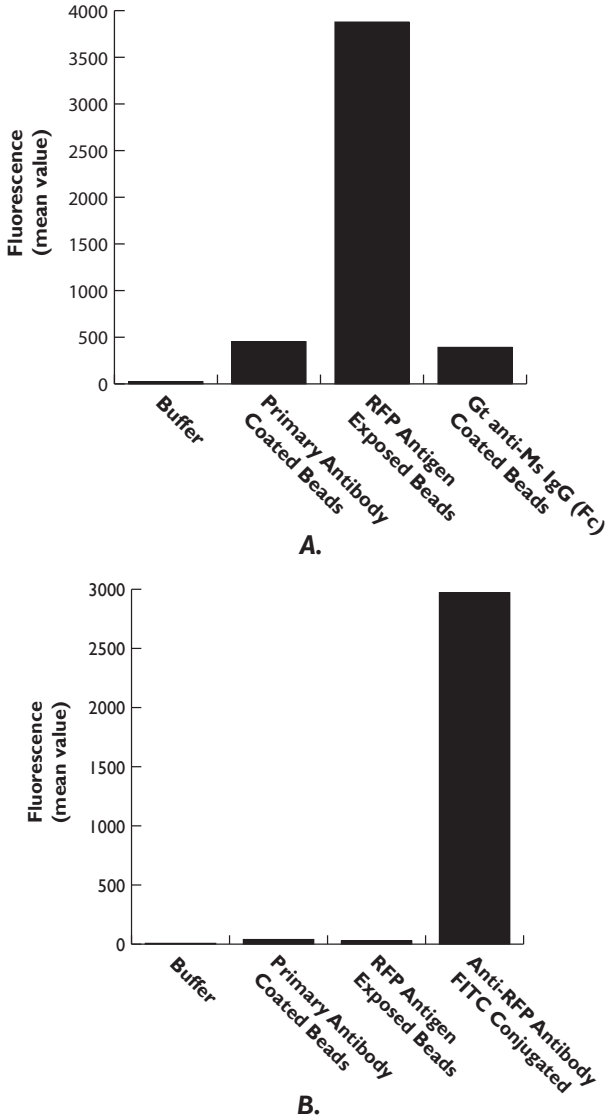
- Goat anti-Mouse IgG (Fc) Coated Polystyrene Particles, 6.28 µm, 0.5% w/v, 5mL, Spherotech, Cat. No. MPFc-60-5
- Primary Antibody: Anti-RFP (Mouse) Monoclonal Antibody, Rockland Immunochemicals, Inc, Cat. No. 200-301-379
- Antigen: Recombinant Red Fluorescent Protein (RFP) Control, Rockland Immunochemicals, Inc, Cat. No. 000-001-379
- Secondary Antibody: Anti-RFP (Rabbit) Antibody Fluorescein Conjugated, Cat. No. 600-402-379
- 1xPBS, pH 7.4

Equipment:

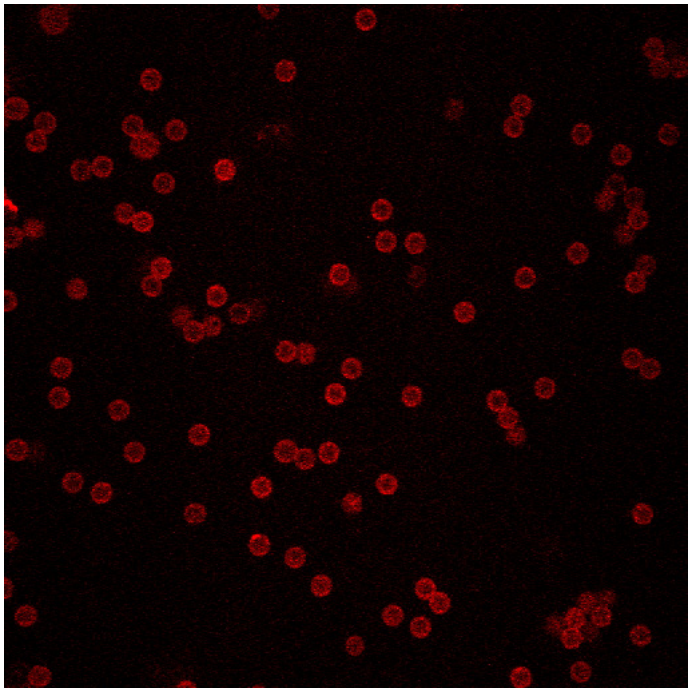
- Eppendorf® Thermomixer®
- Molecular Devices SpectraMAX Gemini
- Nikon C2 Confocal Microscope

Procedure

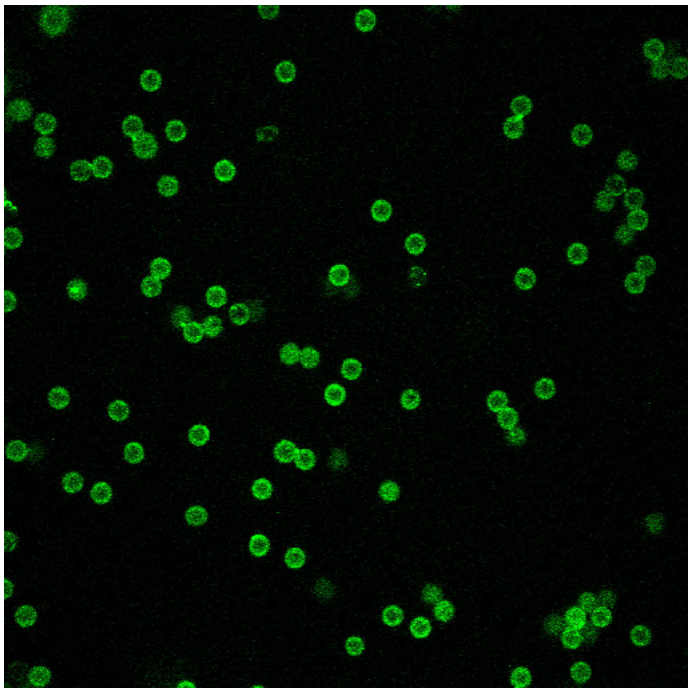
1. Take 200µL of 0.5%w/v Goat anti-Mouse IgG coated beads and concentrate to 100µL of 1.0%w/v by centrifugation at 10 rpm for 10 minutes in a microcetrifuge tube.
2. Add 5µg of the primary antibody to the tube and mix thoroughly using gentle vortexing.
2. Incubate using a microcetrifuge tube bench shaker with gentle mixing for at 1400 rpm for 30 minutes at room temperature.
3. Centrifuge at 1,500x *g* for 20 minutes and remove the supernatant.
4. Wash beads 2 times with 100µL 1x PBS, pH 7.4 to remove excess primary antibody from the bead solution.
5. Add 2µg of the antigen to the primary antibody coated particles. Mix well using a microcetrifuge tube bench shaker with gentle mixing for at 1400 rpm for 30 minutes at room temperature.
6. Wash beads with 100µL 1x PBS, pH 7.4 to remove excess antigen from the bead solution.
7. Add 5µg fluorescent conjugated secondary antibody to the beads and mix well.
8. Incubate using a microcetrifuge tube bench shaker with gentle mixing for at 1400 rpm for 30 minutes at room temperature.
9. Wash beads 2 times with 100µL 1xPBS, pH 7.4 to remove excess secondary antibody from the bead solution.
10. Measure the fluorescence for the RFP antigen at 558ex/583em and for Fluorescein conjugated secondary antibody at 485ex/520em.



Fluorescence data of Gt anti-Ms Coated Beads at the different stages of a sandwich assay. A. 558nm ex/583nm em data showing the binding of RFP antigen to anti-RFP monoclonal primary antibody coated MPFc-60-5 beads. B. 488nm ex/520nm em data of anti-RFP antibody FITC conjugated exposed antigen-primary antibody-MPFc-60-5 bead complex.



A.



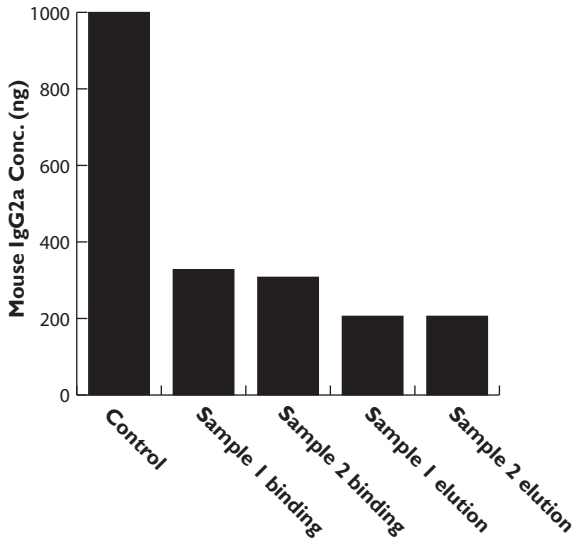
B.

Nikon C2 Images of Goat anti-Mouse Antibody Coated Beads used in a sandwich assay. A. 561nm exciation image of RFP antigen exposed MPFc-60-5 after coating with anti-RFP monoclonal primary antibody. B. 488nm excited image of anti-RFP antibody fluorescein conjugated exposed antigen-primary antibody-MPFc-60-5 bead complex.

Magnetic Immunoprecipitation using Goat anti-Mouse IgG (Fc) Magnetic Beads

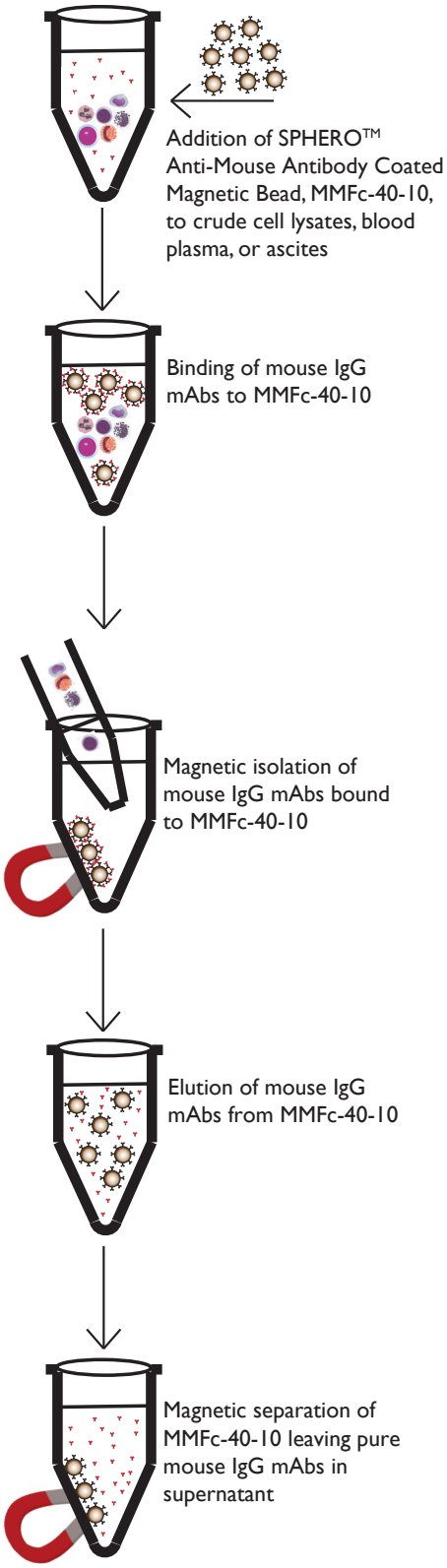
Mouse IgG monoclonal antibody can be purified using Spherotech Cat. No. MMFc-40-10 (Goat anti-Mouse IgG (Fc) Magnetic Particles, 1% w/v, 4.0-5.0µm, 10mL) beads from samples. After IgG binding to MMFc-40-10 beads, elution can be performed by using 0.1M citrate buffer (pH: 2-3) as the elution buffer. Most IgGs will be eluted off at pH 3.1, but the degree of acidity required will depend on the specific IgG antibody.

1. Add 100 µl of 0.1M citrate buffer to the monoclonal IgG coated bead complex.
2. Mix well by tilting and rotation for 2-3 minutes.
3. Place the test tube on a magnet and transfer the supernatant containing purified IgG to a new low protein binding centrifuge tube.
4. Add additional 50 µl 0.1M citrate (pH 2-3) to the bead pellet to elute any remaining IgG.
5. Repeat step 2 and 3.
6. Pipette off the supernatant and pool the supernatants containing pure IgG solutions. At this point, measure the IgG recovered and its purity using UV-Vis at 280nm.



SPHERO™ Goat anti-Mouse IgG (Fc) (100µl, 1% w/v) binding and elution concentrations when exposed to Mouse IgG2a (1000ng)

Immunomagnetic Purification of Mouse IgG mAbs



Regeneration of Spherotech Antibody Coated Beads for Reuse

After elution of the mAb, the Goat anti-Mouse IgG (Fc) Magnetic Particles should be washed three times with PBS and 0.05% BSA and stored at 2-8°C in PBS with BSA.

STORAGE AND STABILITY

If stored unopened at 2-8°C the antibody coated beads are stable until the expiration date stated on a the Certificate of Analysis. This product contains 0.02% sodium azide as a preservative.