SPHERO™ Technical Note **STN-23 Rev A 092820**

SPHERO™ MAGNETIC ANTIBODY COATED PARTICLES FOR CELL ISOLATION

INTRODUCTION

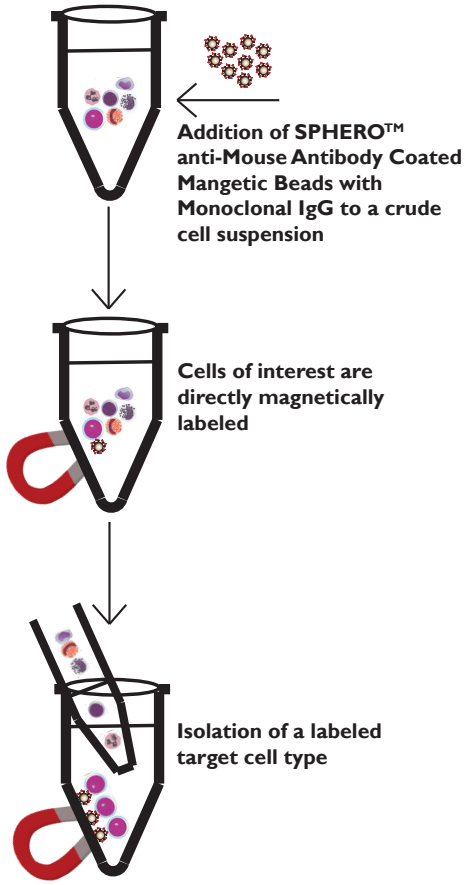
Spheroteh Goat anti-Mouse Magnetic beads coated with a primary mouse IgG antibodies are ideal for isolation of cells from different species (e.g. human, rat) depending on the specificity of the primary antibody. Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions or tissue digests.

Isolation Principle:

There are two methods in cellular isolation. 1. Isolation of required cells from the mixture (positive selection). 2. Capture of unwanted cells from the mixture while the cells of interest remain in the supernatant for studies (negitive selection).

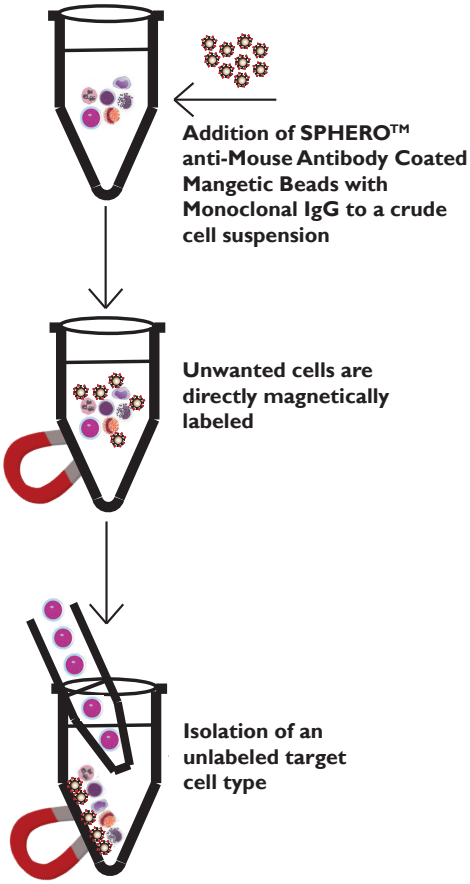
In the most common procedure, the primary antibody or anti cell surface antigen that binds to specific cells is pre-coated onto the beads prior to cell isolation. Antigen coated Goat anti-Mouse magnetic beads such as, Spherotech catalog number MMFc-40-10 are then mixed with the cell sample in a tube. The Goat anti-Mouse magnetic beads with anti cell surface antigen will bind to the target cells to be isolated from the mixture during a short incubation. Afterwards, the bead-bound cells are separated by using elution buffer.

Positive Selection Technique of Cellular Isolation



Example of a cell isolation protocol using SPHERO™ Antibody Coated Magnetic Beads coated with IgG Monoclonal Antibody using the positive selection technique

Negative Selection Technique of Cellular Isolation



Example of a cell isolation protocol using SPHERO™ Antibody Coated Magnetic Beads coated with IgG Monoclonal Antibody using the negitive selection technique

INSTRUCTIONS FOR USE

Materials:

- Spherotech Goat anti-Mouse IgG magnetic beads
- Monoclonal Goat anti-Mouse IgG antibodies
- Buffer for resuspension and isolation: 1X PBS (without Ca2+ and Mg2+)/0.1% BSA /2 mM EDTA, pH 7.4 (filter the buffer if prepared in the lab, commercial buffers can be used without filtering).
- Elution buffer: Chymopapain, 200 U/mL

Beads Washing Procedure: Spherotech Goat anti-Mouse magnetic beads should be washed before use.

1. Resuspend the magnetic beads in the vial.
2. Transfer the desired volume of magnetic beads to a tube.
3. Add the same volume of 1X PBS Buffer, or at least 1 mL, and mix.
4. Magnetically separate and discard the supernatant.
5. Again, resuspend the washed the magnetic beads in the same volume of 1X PBS Buffer and remove the supernatant and resuspend in the original volume with the Buffer for resuspension and isolation.

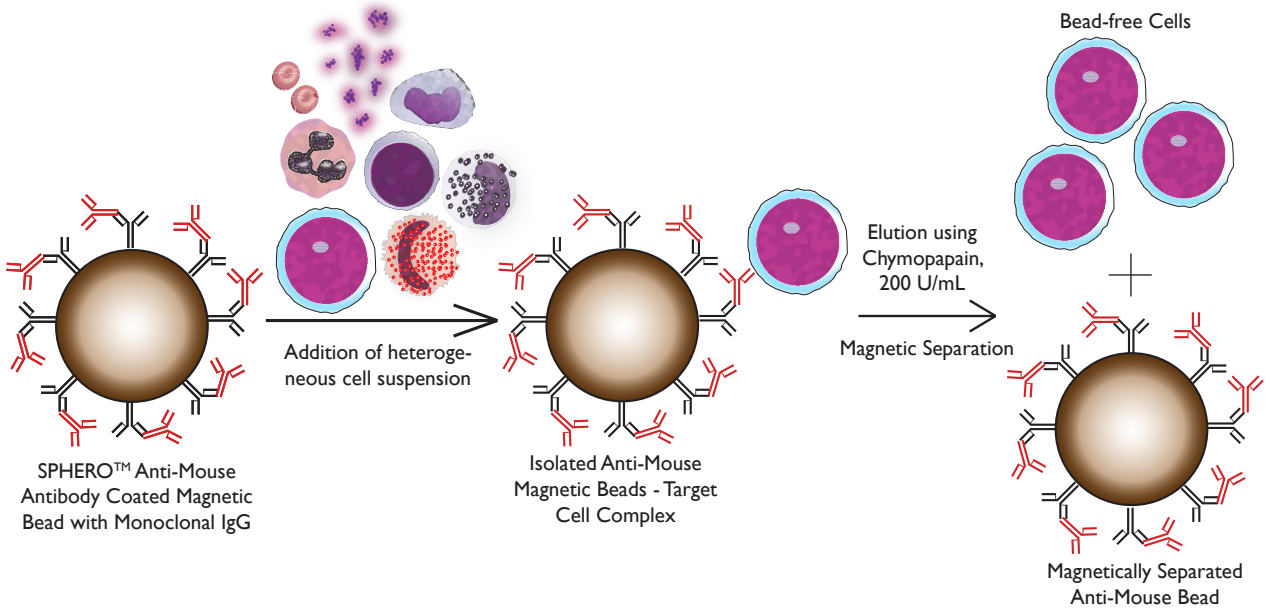
Sample Preparation: Cells can be directly or indirecty isolated from any sample such as whole blood, bone marrow, MNC or tissue digests. Please follow the literature reported sample preparation procedures or custom developed sample preparations.

Direct Isolation Technique:

1. Incubate the heterogeneous mixture of cells with the monoclonal mouse anti-(cell surface antigen or antibody) IgG. Use approximately 10µg of monoclonal antibody / 100 target cells. Allow this incubation to proceed for 30 minutes on ice.
2. Wash to remove unbound antibody from cells
3. Add washed micro particles 10-50 particles/cell to the Heterogeneous cell suspension with antigen
4. Mix gently, incubate cells again for 30 minutes on ice
5. After this incubation, magnetically separate the microspheres and remove the supernatant
6. Optional: Elute cells from microspheres by suspending in elution buffer for 10-30 minutes at 37°C. Magnetically separate and collect supernatant containing cells for further studies.

Indirect Isolation Technique:

1. Add anti surface antigen coated magnetic beads to the prepared heterogenous cell suspension
2. Incubate for 30 min (depletion) at 2 - 8°C with gentle tilting and rotation.
3. Add equal volume of buffer to limit trapping of unbound cells (optional).
4. Magneticially separate and collect the supernatant
5. Transfer the supernatant containing required cells to a fresh tube for further experiments.



Development of a cell isolation protocol using SPHERO™ Antibody Coated Magnetic Beads coated with IgG Monoclonal Antibody using the direct isolation technique

Notes:

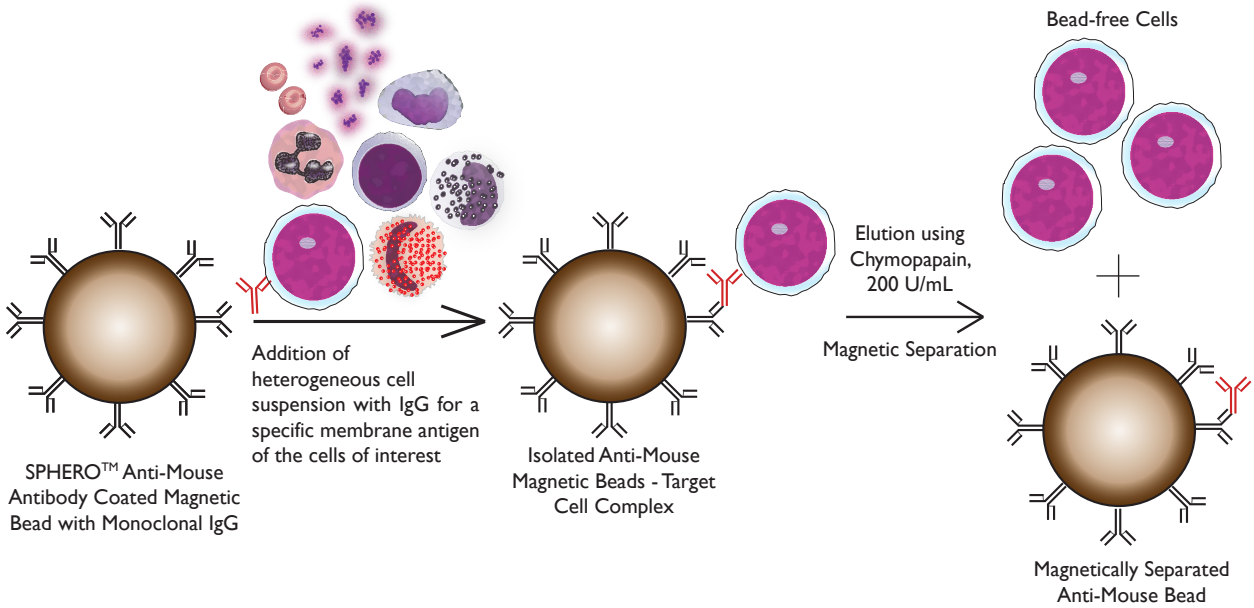
1. Use a mixer that provides tilting and rotation of the tubes to ensure proper mixing of the antibody magnetic beads.
2. When incubating antibody coated beads and cells, the incubation temperature must be 2 - 8°C to reduce phagocytic activity and other metabolic processes.
3. Never use less than 25 µl or 1×10^7 beads/ mL cell sample since it is difficult to handle low volumes or concentrations for binding cells.
4. Chymopapain is a better elution buffer for isolating cells than 0.1 M glycine since it allows microsphere separation without damaging the cells.
5. If the primary antibody has a high affinity use the direct isolation method as seen on pagge 2.
6. If the primary mouse antibodies have a low affinity use the indirect method as seen on page 3.

Antibody Selection

The choice of primary antibody is the most important factor for successful cell isolation. The antibody coated onto goat anti-mouse coated magnetic beads will recognize the heavy chain of most mouse IgG subclasses and is Fc-reactive. Some antibodies may show reduced antigen-binding efficiency when coated onto beads even though the antibody shows good results in other immunological assays. It is important to wash cells prior to adding mouse IgG antibodies or coated magnetic beads. This is due to the soluble factors in serum (e.g. antibodies or cell surface antigens) which can interfere with the cell isolation protocol. Washing the cells once may reduce this interference.

STORAGE AND STABILITY

If stored unopened at 2-8°C upon delivery, the Spherotech Antibody Coated beads are stable until the expiration date stated on the Certificate of Analysis. Keep the magnetic beads in liquid suspension during storage and all handling steps. Drying will result in reduced performance. Resuspend well before use. This product contains 0.02% sodium azide as a preservative which is cytotoxic.



Development of a cell isolation protocol using SPHERO™ Antibody Coated Magnetic Beads coated with IgG Monoclonal Antibody using the indirect isolation technique