*MagCellect™ Streptavidin Ferrofluid**

Catalog Number: MAG999

Reagent Information

- **MagCellect Streptavidin Ferrofluid:** 1 mL in an aqueous solution containing BSA and preservative**. For processing up to 10^9 total cells.**

Storage

Store at 2 - 8° C. **DO NOT FREEZE.**

Intended Use

MagCellect Streptavidin Ferrofluid (SAV-FF) is a colloid of magnetic nanoparticles conjugated to streptavidin. MagCellect SAV-FF is optimized for the isolation of specific cell populations via negative or positive selection using R&D Systems MagCellect Magnet (Catalog # MAG997).

Background

R&D Systems MagCellect technology is based on the use of Ferrofluids (magnetic nanoparticles) that have no magnetic memory. These Ferrofluids have a diameter of ~150 nm and as a result behave like colloidal particles. This allows the Ferrofluids to remain in solution without the need for mixing and additionally allows for efficient diffusion kinetics during the binding reaction. The proprietary manufacturing technology of MagCellect Ferrofluids generates particles with higher ligand binding capacity per mass compared to many other larger diameter magnetic particles.

Principle of Selection

A mononuclear cell suspension is first incubated with a biotinylated antibody from any species or with a cocktail of biotinylated antibodies which target specific cell population(s). MagCellect SAV-FF is next added to the reaction which allows the SAV coated nanoparticles to interact with the cells tagged with the biotinylated antibodies. The tube containing the cell suspension is then placed within a magnetic field. Magnetically tagged cells will migrate toward the magnet, leaving the untagged cells in suspension in the tube. The cells in suspension (negatively selected cells) and the magnetically trapped cells on the wall of the tube (positively selected cells) may be individually harvested depending on the desired target cell population. The enriched cell preparation is then available for a variety of applications including tissue culture, immune status monitoring and flow cytometry.

Cell Selection Procedure

The procedure outlined below is for processing 1 x 10^8 total cells using 12 x 75 mm (5 mL) polystyrene round bottom tubes and the R&D Systems MagCellect Magnet. For processing different cell loads please refer to the Technical Hints section on this insert. Cells and reagents should be kept cold using an ice bath or a refrigerator. **Reaction incubations must be carried out at 2 - 8° C in a refrigerator and not in an ice bath to avoid excessively low temperatures that can slow the kinetics of the optimized reactions**.

1. Prepare reaction buffer ahead of time and keep it on ice or refrigerate until use. Suitable buffers are 1X MagCellect Buffer (prepared by dilution of 10X MagCellect Buffer, R&D Systems, Part # 860040) or phosphate buffered saline supplemented with 0.5% BSA, 2 mM EDTA, and preservative.
2. Prepare a single cell suspension of mononucleated cells from blood or from a lymphoid tissue. To remove cell clumps and/or debris pass the suspended cells through a 40 - 70 µm nylon cell strainer. Cells must be suspended in cold reaction buffer prior to beginning the procedure and be at a density of 1 - 2 x 10^8 cells/mL.
3. **Optional Step:** In some applications a blocking step is required to minimize undesired binding of the antibodies via their Fc domain to the Fc receptors (FcR) present in various cell types. FcR blocking can be achieved by using IgG or specific antibodies that block the Fc receptors. Add 1 - 10 µg of IgG or Fc blocking specific antibody (in a volume not exceeding 100 µL) per 1 x 10^7 cells and incubate 5 - 10 minutes at 2 - 8° C.
4. Transfer 1 x 10^8 cells (0.5 - 1.0 mL volume) into a 5 mL tube. Add up to 100 µL volume of a biotinylated antibody or of a cocktail of biotinylated antibodies. Typically 0.1 - 3.0 µg of each specific antibody per 1 x 10^8 cells is required. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2 - 8° C in a refrigerator for 15 minutes.
5. Add MagCellect SAV-FF to the cell suspension. For **negative selection,** add 75 - 125 µL of SAV-FF per 1 x 10^8 cells. For **positive selection,** add 50 - 100 µL of SAV-FF per 1 x 10^8 cells (the amount to add will vary according to the number of targeted cells and on the density of the surface antigen). Mix gently and incubate at 2 - 8° C in a refrigerator for 15 minutes.

For **negative cell selection,** follow steps 6 - 9.

For **positive cell selection,** follow steps 10 - 13.

Negative Cell Selection:

6. At the end of the incubation period bring the volume of the reaction in the tube to 2 mL by adding reaction buffer. Mix gently to ensure that all reactants in the tube are in suspension.
7. Place the tube in a MagCellect Magnet that has been positioned horizontally to accommodate 5 mL tubes and incubate for 6 minutes at room temperature (18 - 25° C). Magnetically tagged cells will migrate toward the magnet (these are the unwanted cells), leaving the untouched desired cells in suspension in the supernatant.
8. While the tube is in the magnet, using a sterile Pasteur pipette, carefully aspirate all of the reaction supernatant and place it in a new 5 mL tube. Remove the tube containing the magnetically trapped cells from the magnet, and discard.
9. Repeat steps # 7 and # 8 with the new tube containing the recovered cells. The supernatant obtained at the end of these steps is the final depleted cell fraction containing the desired enriched cells. The cells are now ready for counting and further downstream applications.

Positive Cell Selection:

10. At the end of the incubation period transfer the cell suspension to a 50 mL conical centrifuge tube, wash the cells with 15 volumes of reaction buffer and centrifuge at 300 x g for 8 minutes. Pipette out the supernatant completely, resuspend the cell pellet in 2 mL of reaction buffer and transfer the cell suspension to a new 5 mL tube.
11. Place the tube in a MagCellect Magnet that has been positioned horizontally to accommodate 5 mL tubes and incubate for 6 minutes at room temperature (18 - 25° C). Magnetically tagged cells will migrate toward the magnet (these are the desired cells), leaving the unwanted cells in suspension.
12. While the tube is in the magnet, using a sterile Pasteur pipette, carefully aspirate all of the reaction supernatant and discard it. Remove the tube containing the desired magnetically trapped cells from the magnet and resuspend these cells in 2 mL of reaction buffer by gently pipetting up and down.
13. Repeat steps #11 and # 12 with the positively selected cell fraction following cell resuspension. The resultant cell fraction obtained at the end of these steps contains the desired enriched cells. The cells are now ready for counting and further downstream applications.

Technical Hints

- If sterile cells are required following the cell selection, the entire procedure should be carried out in a laminar flow hood to maintain sterile conditions. Use sterile equipment when pipetting.
- Avoid antibody capping on cell surfaces and non-specific cell tagging by working fast, keeping cells and solutions cold through the use of pre-cooled solutions and by adhering to the incubation times and temperatures specified in the protocol. Increased temperature and prolonged incubation times may lead to non-specific cell labeling thus lowering cell purity and yield.
- When processing 2 x 10^8 cells or fewer, use the 12 x 75 mm (5 mL) tubes with the MagCellect Magnet horizontally positioned to accommodate up to six 5 mL tubes. **Do not process more than 2 x 10^8 cells in each 5 mL tube and do not exceed a total reaction volume of 3 mL in each tube**. A reaction volume of 3 mL is recommended for processing 2 x 10^8 cells. A reaction volume of 1 mL is recommended when processing 5 x 10^7 or fewer cells. **Reaction volume adjustments must be made using reaction buffer just prior to the magnetic separation step.**
- When processing greater than 2 x 10^8 cells, use 17 x 100 mm (15 mL) polystyrene round bottom tubes with the MagCellect magnet vertically positioned to accommodate up to two 15 mL tubes. **Do not process more than 6 x 10^8 cells in each 15 mL tube and do not exceed a total reaction volume of 9 mL in each tube.** When using this larger tube, increase the reaction volume before the magnetic separation step according to the following formula: 3 mL for each 2 x 10^8 cells processed. Also increase the magnetic incubation times to 8 minutes. **Reaction volume adjustments must be made using reaction buffer just prior to the magnetic separation step.**