MagCellect™ Mouse Hematopoietic Cell Lineage Depletion Kit

Catalog Number: MAGM209

INTENDED USE

The MagCellect Mouse Hematopoietic Cell Lineage Depletion Kit is designed for the enrichment of hematopoietic non lineage committed cells (lineage negative) from mouse bone marrow. Lineage committed cells (lineage positive) targeted for magnetic depletion include T cells, B cells, NK cells, monocytes/macrophages, granulocytes, and erythrocytes. The resulting cell population is highly enriched for $CD117^+$ cells (from 40-70% depending on mouse strain) with less than 5% residual lineage positive cells.

BACKGROUND

R&D Systems MagCellect products are designed for the isolation of cells in a "liquid phase". MagCellect technology is based on the use of ferrofluids or magnetic nanoparticles that have no magnetic memory (superparamagnetic) and behave like colloidal particles. This feature 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

Isolation of lineage negative cells is carried out in a test tube and is achieved through magnetic removal of lineage positive cells. Lineage positive cells are removed by use of the MagCellect Mouse Cell Lineage Depletion Biotinylated Antibody Cocktail followed by the addition of Streptavidin Ferrofluid (SAV-FF). The tube with the cell suspension is then placed in the MagCellect Magnet; magnetically tagged cells will migrate toward the tube wall on the magnet side (unwanted cell fraction or lineage positive), leaving the untagged cells or desired cell population (lineage negative) in suspension. While the tube remains in the magnet, the cells can be harvested by aspiration.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date. **DO NOT FREEZE.**
The kit contains sufficient reagents to process $1x10^9$ total cells.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Mouse Cell Lineage Depletion Biotinylated Antibody Cocktail	860058	1 mL of a phosphate buffered solution containing BSA.	May be stored 2-8 °C when handled aseptically.*
Blocking Reagent-1	860059	0.5 mL of a phosphate buffered solution containing BSA and rat IgG.	
Streptavidin Ferrofluid	860128	1.5 mL of a solution containing BSA and preservative.	
10X Buffer	860125	25 mL of a 10X concentrated buffer.	May be stored for up to 24 hours at 2-8 °C after dilution*

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- MagCellect Magnet (R&D Systems, Catalog # MAG997)
- 12 x 75 mm (5 mL) or 17 x 100 mm (15 mL) polystyrene round bottom tubes
- Sterile Pasteur pipettes or transfer pipettes (ThermoFisher, Catalog # 13-711-9B) or equivalent
- Sterile deionized or distilled water
- Rat anti-mouse CD117-PE (R&D Systems, Catalog # FAB1356P), rat IgG2A -PE (R&D Systems, Catalog # IC006P), and Streptavidin-PE (R&D Systems, Catalog # F0040).

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PRECAUTION

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

REAGENT PREPARATION

Prepare 25 mL of 1X MagCellect Buffer for each 1 x 10^8 cells to be processed by mixing 2.5 mL of 10X Buffer with 22.5 mL sterile deionized or distilled water. Must be kept at cold (2-8 °C) for procedure.

CELL PREPARATION

1. Prepare a mononuclear suspension of mouse bone marrow (BM) cells using traditional methods (*Current Protocols in Immunology, Isolation of Murine Macrophages*, copyright 1994, Coligan, J.E. *et al.* eds. John Wiley & Sons, Inc., Volume 3, Supplement 11, 14.1.4.).
2. To remove cell clumps and debris after harvesting the BM cells, pass the cell suspension through a 70 µm nylon cell strainer (Falcon, Catalog # 352350 or equivalent).
3. Wash cells in 50 mL centrifuge tubes with cold 2% FBS-PBS by centrifuging at 300 x g for 8 minutes at 2-8 °C.
4. Remove supernatant completely and resuspend the cells in 2 mL of cold 1X MagCellect Buffer.
5. Count the cells, and bring the cell suspension to 2 x 10^7 cells/mL with 1X MagCellect Buffer. **Lysing of red blood cells is not required.**

CELL LINEAGE DEPLETION PROCEDURE

This procedure describes the processing of 1 x 10^8 total cells. For processing other cell numbers please refer to the Technical Hints section on this insert. Both femurs and tibiae from one mouse typically yield 2-6 x 10^7 hematopoietic cells; of these 0.3-1.0 x 10^6 are lineage negative cells. Reagents and cells should be kept cold using an ice bath or a refrigerator until use. **Reaction incubations must be carried out at 2-8 °C in a refrigerator and not in an ice bath in order to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

1. Place 1 x 10^8 cells (5.0 mL volume) into a sterile 15 mL centrifuge tube. Add 50 µL of Blocking Reagent-1. mix gently and incubate at 2-8 °C in a refrigerator for 15 minutes.
2. Add 100 µL of Mouse Cell Lineage Depletion Biotinylated Antibody Cocktail. Mix gently and incubate at 2-8 °C in a refrigerator for 15 minutes.
3. Wash away excess antibody by filling the tube to the 15 mL mark with **cold** 1X MagCellect Buffer and centrifuge at 300 x g for 8 minutes.
4. Remove the supernatant completely and resuspend the cell pellet with 1 mL of **cold** 1X MagCellect Buffer by gently pipeting up and down.
5. Transfer the cells to a clean 5 mL tube (12 x 75 mm). Retain 5 x10^5 cells to assess the proportion of lineage positive cells by staining with a suitable Streptavidin-fluorochrome conjugate.
6. Add 150 µL of Streptavidin Ferrofluid. Mix gently and incubate at 2-8 °C in a refrigerator for 15 minutes.
7. At the end of the incubation period bring the volume of the reaction in the tube to 2 mL by adding 0.85 mL of 1X MagCellect Buffer. Mix gently to ensure that all reactants in the tube are in suspension.
8. Place the reaction tube in the MagCellect Magnet that has been positioned horizontally to accommodate 5 mL tubes and incubate for 6 minutes at room temperature. Magnetically tagged cells will migrate toward the magnet (these are the unwanted cells), leaving the untouched desired cells in suspension in the supernatant.
9. Recovery of desired cells is achieved as follows: While the tube is in the magnet, using a sterile Pasteur pipette or transfer 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.
10. To ensure that all of the magnetic nanoparticles have been removed, repeat the magnetic depletion (steps 8 and 9) 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 lineage negative cells. The cells are now ready for counting, staining or other 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 reagents that will be reused at a later date.
- Avoid antibody capping on cell surfaces and non-specific cell tagging by working quickly, 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 different numbers of cells observe the following guidelines: keep antibody cocktail and ferrofluid incubation times and temperatures the same; keep the cell density at 1 x 10^8 cells/mL; add 10 µL of the antibody cocktail per 1 x 10^7 cells being processed; add 12.5 µL of Streptavidin Ferrofluid per 1 x 10^7 cells being processed.
- 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 2 mL is recommended for processing 1 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 1X MagCellect Buffer just prior to the magnetic separation step.**
- When processing greater than 2 x 10^8 cells, use the 17 x 100 mm (15 mL) 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 time described in step #8 to 8 minutes. **Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.**

DATA EXAMPLE

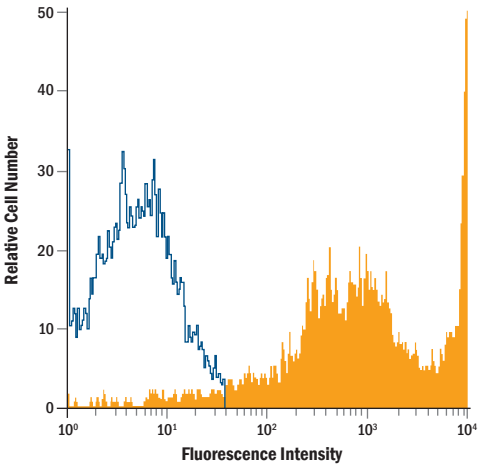

Figure 1: Lineage marker reactivity on BALB/c bone marrow (BM) cells processed with this kit. Histograms show reactivity of BM cells labeled with the cocktail of biotinylated antibodies before (filled) and after (open) magentic depletion. Lineage marker reactivity was developed by using Streptavidin-PE.

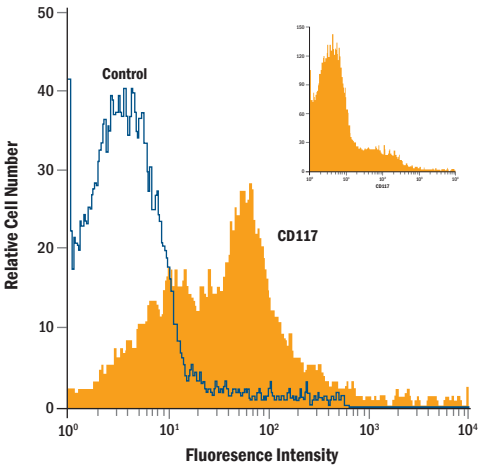

Figure 2: CD117 (c-kit) staining of bone marrow lineage negative cells isolated from BALB/c mice using this kit. Histograms reflect reactivity of all viable cells with CD117-PE or a matched isotype control. Inset shows CD117 staining of cells before isolation.