MagCellect™ Human Eosinophil Isolation Kit*

Catalog Number: MAGH117

INTENDED USE

This MagCellect™ Human Eosinophil Isolation Kit is designed to isolate eosinophils via a negative selection principle. The resulting cell preparation is highly enriched with eosinophils. Typical purity of recovered eosinophils ranges between 80% and 95%.

PRINCIPLE OF SELECTION

A cell suspension is first incubated with the MagCellect Human Eosinophil Biotinylated Antibody Cocktail which targets unwanted cells. MagCellect Streptavidin Ferrofluid is added to the reaction and the streptavidin-coated nanoparticles interact with the biotinylated antibody tagged cells. The tube containing the cell suspension is then placed within a magnetic field. Magnetically tagged cells will migrate toward the magnet (unwanted cell fraction), leaving the untagged cells or desired cell population in suspension to be harvested by aspiration while the tube remains in the magnetic field. The enriched cell preparation is then available for a variety of applications including tissue culture, immune status monitoring, and flow cytometry.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. **DO NOT FREEZE.**
This kit contains sufficient reagents to process 1 x 10^9 total cells; up to 25 isolations.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Human Eosinophil Biotinylated Antibody Cocktail	860205	2.0 mL of a phosphate buffered solution containing BSA.	May be stored at 2-8 °C when handled aseptically.*
Streptavidin Ferrofluid	860127	2.0 mL of a solution containing BSA and preservatives.	
10X Buffer 2	860206	10 mL of a 10-fold 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) or equivalent*
- 12 x 75 mm (5 mL) polystyrene round bottom tubes
- Sterile Pasteur pipettes or transfer pipettes (ThermoFisher, Catalog # 13-711-9B) or equivalent

SUPPLIES REQUIRED IF USING CELL PREPARATION SECTION BELOW

- Human Erythrocyte Lysing Kit (R&D Systems, Catalog # WL1000 or equivalent)

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.

CELL PREPARATION

Use this procedure or traditional methods to prepare a single cell suspension of human polymorphonuclear granulocytes.

1. Collect whole blood into tubes with anticoagulant using the method of choice.
2. Transfer 25 mL aliquots of blood into 50 mL conical tubes, add 1X PBS to the 50 mL mark and mix by inverting the tubes two times. Centrifuge at 350 x g for 10 minutes, and remove the supernatant.
3. Add 1X PBS to the 30 mL mark. Invert the tubes several times to mix well.
4. In 50 mL conical tubes (as many tubes as aliquots), add 20 mL of Ficoll-Paque® at room temperature.
5. Carefully layer the 30 mL of blood on the Ficoll to perform a standard density separation.
6. Centrifuge at 650-700 x g for 40 minutes. Carefully remove and discard the plasma layer, mononuclear cells ('buffy coat'), and the Ficoll. Keep the pellet (containing granulocytes and erythrocytes).
7. Add R&D Systems 1X Human Erythrocyte Lyse Solution to the 50 mL mark. Invert a few times to mix well. If the RBC layer is above the 15 mL mark after centrifuging, split the tubes in order to lyse thoroughly. Incubate for 10-15 minutes at room temperature.
8. Centrifuge at 350 x g for 10 minutes. Remove the supernatant and break the pellet. Wash the cells by adding 1X PBS up to the 50 mL mark. Invert the tubes to mix well. Centrifuge at 350 x g for 10 minutes.
9. Remove the supernatant, break up the pellet, and resuspend in 1X PBS. Centrifuge at 350 x g for 10 minutes.
10. Remove the supernatant, break up the pellet, and resuspend in 1X MagCellect Buffer 2 to a concentration of at least 1 x 10^8 cells/mL.

CELL SELECTION PROCEDURE

This procedure is for processing of 1 x 10^8 total cells using 5 mL tubes and the MagCellect Magnet. For processing other cell numbers, please refer to the Technical Hints section. 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 10 mL of 1X MagCellect Buffer 2 for each 1 x 10^8 cells to be processed by mixing 1 mL of 10X Buffer 2 with 9 mL of sterile deionized or distilled water. The 1X buffer should be kept on ice or refrigerated and used within 1 month.
2. Prepare a single cell suspension of granulocytes by traditional methods or by following the instructions outlined in the Cell Preparation section. Cells must be suspended in cold 1X MagCellect Buffer 2 prior to beginning the procedure and be at a cell density of 1 x 10^8 cells/mL.
3. Transfer 1 x 10^8 cells (1 mL volume) into a 5 mL polystyrene tube. Add 200 µL of Human Eosinophil Biotinylated Antibody Cocktail. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2-8 °C in a refrigerator for 15 minutes.
4. Add 250 µL of Streptavidin Ferrofluid to the cell suspension, mix gently, and incubate at 2-8 °C in a refrigerator for 15 minutes. Avoid bubble formation.
5. At the end of the incubation period, bring the volume of the reaction in the tube to 3 mL by adding 1.55 mL of 1X MagCellect Buffer 2. Mix gently to ensure that all reactants in the tube are in suspension.
6. 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.
7. Recovery of the desired cells is achieved as follows: While the tube is firmly held in the magnet, use a sterile Pasteur pipette or transfer pipette to slowly aspirate all of the reaction supernatant into a new 5 mL tube. Remove the tube containing the magnetically trapped cells from the magnet, and discard.
8. To ensure that all of the magnetic nanoparticles have been removed, repeat the magnetic depletion (steps 6 and 7) with the new tube containing the recovered cells. Avoid bubble formation. The supernatant obtained at the end of these steps is the final depleted cell fraction containing the desired enriched eosinophils. 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 reagents that will be reused at a later date.
- Avoid antibody capping on cell surfaces and non-specific cell tagging by working fast, keeping cells and solutions cold throughout 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.
- While the procedure outlined in the Cell Selection Procedure is strongly recommended, follow the recommendations in Table 1 or observe the following guidelines when processing different numbers of cells: 1) Keep the Antibody Cocktail and the Streptavidin Ferrofluid incubation times and temperatures the same; 2) Keep the cell density at 1 x 10^8 cells/mL; 3) Add 20 µL of the Antibody Cocktail per 1 x 10^7 cells being processed; 4) Add 25 µL of Streptavidin Ferrofluid per 1 x 10^7 cells being processed. A minimum of 75 µL and a maximum of 400 µL of Streptavidin Ferrofluid is required per isolation.

Recommended quantities to be used in steps 3-4 of the Cell Selection Procedure (100 X 10^6 is recommended).

Number of Cells in Starting Preparation	5 x 10^7	1 x 10^8	2 x 10^8
Reaction Volume	1 mL	**1 mL**	2 mL
Human Eosinophil Biotinylated Antibody Cocktail	100 µL	**200 µL**	400 µL
Streptavidin Ferrofluid	100 µL	**250 µL**	400 µL

- 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 1 mL is recommended for processing 1 x 10^8 cells or less. A reaction volume of 2 mL is recommended when processing 2 x 10^8. **Reaction volume adjustments must be made using 1X MagCellect Buffer 2 just prior to the magnetic separation step.**

DATA EXAMPLES

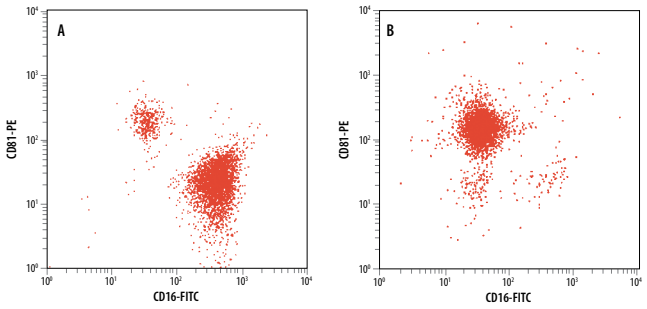

Figure 1: Enrichment of eosinophils from whole blood using this MagCellect Human Eosinophil Isolation Kit. Cells, before **(A)** and after **(B)** enrichment, were double-stained with PE-conjugated anti-human CD81 antibody (R&D Systems, Catalog # FAB4615P) and fluorescein conjugated anti-human CD16 (R&D Systems, Catalog # FAB2546F) and analyzed by flow cytometry. Before MagCellect isolation, as observed, cells are predominantly $CD16^+/CD81^-$ non-eosinophil granulocytes (A), while isolation of eosinophils with this kit results in significant enrichment of $CD16^-/CD81^+$ (B).

TROUBLESHOOTING GUIDE

Most difficulties arise from the following areas:

Quality of the Cell Preparation: Dead cells and debris might interfere with isolation and detection, affecting both the purity and recovery of intended cells. Cell aggregation might also affect the enrichment performance.

Few Expected Target Cells: If less than 100,000 expected target cells are present, recovery and/or purity of the isolation is compromised.

Issue	Possible Cause	Possible Solution
Low yield of isolated cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris will affect the isolation efficiency. Make sure your cell preparation contains a minimal amount of dead cells or cell debris. Test a small sample of cells with a vital dye before performing the cell selection procedure. The presence of cell debris is also easily identified in the FSS/SSC flow cytometry analysis.
	Cell aggregates	Cell aggregates will interfere with the cell selection. Make sure you have a single-cell suspension before performing the cell selection procedure. A small sample of cells can be tested with a vital dye before performing the cell selection procedure to ensure a healthy single-cell suspension.
	Few expected cell targets	If the cell fraction to be isolated contains less than ~250,000 cells or represents less than 1% of the total cell preparation, recovery could be affected. For a better yield, increase the number of cells in your starting population, if possible, or consider performing a pre-enrichment step by prior removal of known undesirable cells in your preparation (R&D Systems has MagCellect kits for negative selection of undesirable cells).
	Poor magnetic selection	When removing unwanted cells in step 7 of the Cell Selection Procedure, make sure the tube in the magnet does not move. If the tube is allowed to move or shift, cells that should be magnetically attached to the magnet might become loose. If the placement of the tube in the magnet is not tight, immobilize it with adhesive tape. Also, be sure to aspirate the supernatant very carefully when removing the unwanted cells. Strong pipetting might release undesired cells from the magnet.
Low purity of isolated cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris will affect the isolation efficiency. Make sure your cell preparation contains a minimal amount of dead cells or cell debris. Test a small sample of your cells with a vital dye before performing the cell selection procedure. The presence of cell debris could also be easily identified in the FSS/SSC flow cytometry analysis.
	Few positive cell targets	If the cell fraction to be isolated contains less than ~250,000 cells or represents less than 1% of the total cell preparation, recovery could be affected. For a better yield, increase the number of cells in your starting population, if possible, or consider performing a pre-enrichment step by prior removal of known undesirable cells in your preparation (R&D Systems has MagCellect™ kits for negative selection of undesirable cells).
	Enriched cells not washed well	Extra washes can be performed subjecting the cells to an extra step of magnetic migration (steps 7-8 of the Cell Selection Procedure). Additional magnetic selection steps could increase cell purity (typically ~5% increase) of the target population. Keep in mind that with every added step a reduced yield can be expected.
No cells recovered	Insufficient cell targets	If the cell fraction to be isolated represents a very small fraction of the total cell preparation, recovery could be significantly reduced. For a better yield, increase the number of cells in your starting population and/or consider performing a pre-enrichment step by removing undesirable cells (R&D Systems has MagCellect kits for negative selection of undesirable cells).