Product Insert: T-cell Positive Selection Reagent Kit for use with the FerroSelect™ QP5 Quadrupole Magnet

For Research Use Only

Sufficient materials are included in the kit to allow the separation of $CD3^+$ T cells from approximately 2.4 x 10^8 peripheral blood mononuclear cells (PBMC). The maximum number of PBMC employed per separation in the FerroSelect™ QP5 Quadrupole magnet is 8.0 x 10^7 cells.

Product Description:

1.0 mL of 12 µg/mL biotinylated anti-CD3 monoclonal antibody in PBS containing 1.0% recombinant human serum albumin (rHSA).

1.0 mL of 75 µg/mL streptavidin ferrofluid (SA-FF) in 0.3% rHSA.

Storage: Store at 2 – 8 °C. **Do Not Freeze**.

Expiry Date as per label/CoA (contact quality@biomagneticsolutions.com for updates).

Product Applications:

Positive selection of $CD3^+$ cells from apheresis products, PBMC, or cell culture suspensions. The isolated cells can be used for further analysis, assays, and expansion studies.

BioMagnetic Solutions have used fresh (non-frozen) cellular products for method development. Customers using frozen products such as cord blood for cell selection studies should develop their own procedures.

Additional Recommended Materials:

The items below are used to produce the buffer employed in $CD3^+$ cell selection studies: Phosphate Buffered Saline (minus Ca^{2+} and Mg^{2+} with 2.0 mM Ethylene-diamino-tetraacetic acid (EDTA)) containing 1.0% HSA (PBS-HSA).

- Phosphate buffered saline (minus Ca^{2+} and Mg^{2+}) including 2.0 mM EDTA supplied either separately by BioMagnetic Solutions (Cat. No: 14-00005, 14-0007) or produced by the end user.
- Human Serum Albumin (25% HSA, Akron, Cat. No: AK8228-0100 or equivalent depending upon end user requirements).

Cellular products employed in $CD3^+$ selection studies should be washed and resuspended in the above buffer at a concentration of 2.0 x 10^8 cells/mL.

5 mL tube to be used with the QP5 quadrupole (12 x 75 mm tube).

FerroSelect™ QP5 Quadrupole Magnet – Cat. No: 24-0001

Human IgG (H-IgG). This is used to block non-specific binding.

Alternatives to H-IgG should be validated by end user.

Safety:

Wear gloves, a lab coat, and safety glasses at all times when handling reagents and blood products.

Cells selected using the RUO reagent kit are **not** for human use.

Users have a ‘duty of care’ to dispose of all biological waste safely in accordance with biomedical waste guidelines.

Warranty:

BioMagnetic Solutions does not offer any warranty regarding the performance of the reagent kit due to the variability of the starting product employed for cell selections.

The kit should not be used after its expiry dating.

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Procedure

The following procedure was developed by BioMagnetic Solutions' Research and Development Department as a guide to the end user. Approximately 8.0 x 10^7 PBMC were employed as the starting product per the experiment described below, and the cell separations were undertaken using a 5 mL (12 x 75 mm) tube fitting into the FerroSelect™ QP5 Quadrupole magnet.

1. Cell Preparation

The starting celular product should be washed twice in PBS-HSA (buffer), and resuspended to a concentration of 2.0 x 10^8 cells/mL (0.4 mL) using a 5 mL tube.

If the sample contains monocytes, Human IgG (H-IgG) should be added to a final concentration of 1.0 mg/mL (50 µL) to block nonspecific binding. The incubation volume will be 0.45 mL. Cells should be incubated after gentle mixing for approximately 5 minutes at room temperature.

2. Antibody Labeling

Approximately 150 µL of buffer should be added to the cell mixture before adding the biotinylated mAb.

The vial of biotinylated anti-CD3 mAb should be gently mixed and 200 µL of antibody added to the tube containing the cells. The tube's contents should be mixed gently by pipetting. The incubation volume will be 0.80 mL.

Cells should be incubated for 5 minutes at room temperature.

3. Ferrofluid Labeling

Approximately 544 µL of buffer should be added to the tube before adding SA-FF.

The vial of streptavidin ferrofluid should be gently mixed by inversion and 256 µL added to the cell suspension **without** washing excess mAb from the cellular product. The tube should be mixed gently by pipetting. The incubation volume will now be 1.6 mL.

Cells should be incubated for 5 minutes at room temperature.

4. Cell Separation

After incubation, 2.4 mL of buffer should be added to the tube containing the cell suspension and gently mixed.

The tube should be inserted into the quadrupole magnet with a separation time of 10 minutes to allow cells labeled with SA-FF to be drawn to the walls of the tube.

The supernatant should be carefully aspirated with a Pasteur pipette from the tube without touching the tube's sides.

The tube should be removed from the Quadrupole magnet and 4.0 mL of buffer added with gentle mixing to resuspend the cellular product.

The tube should be inserted into the Quadrupole magnet and allowed to separate for an additional 10 minutes to capture cells labeled with SA-FF. After draining the supernatant from the tube and removing it from the magnet, cells should be resuspended in buffer for further investigations.

NOTE: depending on the end user needs, cells may be washed more than once to increase the purity of the $CD3^+$ cellular product.

5. Expected Results

BioMagnetic Solutions have used this protocol to obtain a mean purity of $CD3^+$ cells of 91% and a mean yield of 55% (datasheets available on request), although the end user should be aware that variations in the starting product will impact the results obtained.