

Sufficient materials are included in the kit to allow the separation of CD3⁺ T cells from approximately 2.4 x 10⁸ peripheral blood mononuclear cells (PBMC). The maximum number of PBMC employed per separation in the FerroSelect™ QP15 Quadrupole magnet is 2.4 x 10⁸ cells.

Product Description:

1.0 mL of 12 µg/mL biotinylated anti-CD3 monoclonal antibody (mAb) 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 Ca²⁺ and Mg²⁺ with 2.0 mM Ethylene-diamino-tetraacetic acid (EDTA)) containing 1.0% HSA (PBS-HSA).

- Phosphate buffered saline (minus Ca²⁺ and Mg²⁺) 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 10⁸ cells/mL.

15 mL tube to be used with the QP15 quadrupole (17 x 120 mm tube).

FerroSelect™ QP15 Quadrupole Magnet – Cat. No: 24-0002

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.

Procedure

The following procedure was developed by BioMagnetic Solutions' Research and Development Department as a guide to the end user. Approximately 2.4×10^8 PBMC were employed as the starting product per the experiment described below, and the cell separations were undertaken using a 15 mL (17 x 120 mm) tube fitting into the FerroSelect™ QP15 Quadrupole magnet.

1. Cell Preparation

The starting cellular product should be washed twice in PBS-HSA (buffer), and resuspended to a concentration of 2.0×10^8 cells/mL (1.2 mL) using a 15 mL tube. If the sample contains monocytes, Human IgG (H-IgG) should be added to a final concentration of 1.0 mg/mL (130 μ L) to block nonspecific binding. The incubation volume will be 1.33 mL. Cells should be incubated after gentle mixing for approximately 5 minutes at room temperature.

2. Antibody Labeling

Approximately 470 μ L 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 600 μ L of antibody added to the tube containing the cells. The tube's contents should be mixed gently by pipetting. The incubation volume will be 2.4 mL.

Cells should be incubated for 5 minutes at room temperature.

3. Ferrofluid Labeling

Approximately 1.63 mL of buffer should be added to the tube before adding SA-FF.

The vial of streptavidin ferrofluid should be gently mixed by inversion and 770 μ L 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 4.8 mL.

Cells should be incubated for 5 minutes at room temperature.

4. Cell Separation

After incubation, 7.2 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 12.0 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

Using the QP5, 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. A smaller number of experiments have been undertaken with the QP15 magnets that show equivalent performance to the QP5.