

Product Description:

Positive selection of CD8⁺ T cells from apheresis products, peripheral blood mononuclear cells (PBMC), or cell culture suspensions using BioMagnetic Solutions FerroSelect™ Quadrupoles (QPs). The isolated cells can be used for further analysis, assays, and expansion studies.

IMPORTANT NOTE: BioMagnetic Solutions 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. Suggestions for using our products with frozen starting materials is available. Please contact us for assistance.

Sufficient materials are included in the kit to allow the separation of CD8⁺ T cells from approximately 2.4 – 4.8 x 10⁸ PBMC.

| Product Contents | |
|--------------------------------------|---|
| Biotinylated anti-CD8 mAb | 1 vial: 1.0 mL, 5 µg/mL in PBS w/ 1.0% rHSA |
| Streptavidin Ferrofluid (SA-FF) | 1 vial: 1.0 mL, 75 µg/mL in 0.3% rHSA |
| Storage: 2-8 °C Do Not Freeze | Expiry Date: As per label/CoA |

rHSA – recombinant Human Serum Albumin, mAb – Monoclonal Antibody

BioMagnetic Solutions Required Products:

FerroSelect Quadrupole:

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

OR

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

Additional Required Materials:

The items below are used to produce the buffer employed in CD8⁺ cell selection studies:

Phosphate Buffered Saline (minus Ca²⁺ and Mg²⁺) with 2.0 mM EDTA containing 1.0% HSA (PBS-HAS)

- Phosphate buffered saline (minus Ca²⁺ and Mg²⁺) including 2.0 mM EDTA supplied either separately by BioMagnetic Solutions or produced by the user
 - Recommended PBS: Corning PBS (Cat. No: 21-0031-CV) or equivalent
 - Recommended EDTA: Fisher Scientific (Cat. No: S311) or equivalent
- Human Serum Albumin
 - Recommended HSA: 25% HSA, Akron, (Cat. No: AK8228-0100) or equivalent

Disposable Tube for use with the Quadrupole:

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

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

Human IgG (H-IgG): This is used to block non-specific binding. Recommended H-IgG: Lampire (Cat. # 7403704, 10 mg/mL). Alternatives to H-IgG should be validated by the user.

Procedure:

The following procedure was developed by BioMagnetic Solutions' Research and Development Department as a guide to the user. Follow the column for the appropriate Quadropole.

| | | QP5 Quadropole Selection | QP15 Quadropole Selection |
|-------------------------------|--|--|---|
| Application: | | Separation of $0.8 - 1.6 \times 10^8$ PBMC using a 12 x 75 mm tube | Separation of $2.4 - 4.8 \times 10^8$ PBMC using a 17 x 120 mm tube |
| 1. Cell Preparation | | | |
| 1.1 | Wash Cells | Wash cells by centrifugation 2x in PBS-HSA | |
| 1.2 | Resuspend Cells | Resuspend to $2.0 - 4.0 \times 10^8$ cells/mL and aliquot 0.4 mL into a fresh tube | Resuspend to $2.0 - 4.0 \times 10^8$ cells/mL and aliquot 1.2 mL into a fresh tube |
| 1.4 | <i>Add Human IgG (H-IgG) to block nonspecific binding.</i> | Add H-IgG to the cells at a final concentration of 1.0 mg/mL | |
| 1.5 | | Add 80 μ L of recommended H-IgG (Volume 0.48 mL) | Add 240 μ L of recommended H-IgG (Volume 1.44 mL) |
| 1.6 | | Incubate after gentle mixing for 5 min at RT | |
| 2. Antibody Labeling | | | |
| 2.1 | Dilute | Add 160 μ L of PBS-HSA to cell mixture | Add 480 μ L of PBS-HSA to cell mixture |
| 2.2 | Mix | Gently mix the vial of anti-CD8 mAb | |
| 2.3 | Add mAb | Add 160 μ L mAb to the cells, gently mix (Volume 0.80 mL) | Add 480 μ L mAb to the cells, gently mix (Volume 2.4 mL) |
| 2.4 | Incubate | Incubate for 5 minutes at RT | |
| 3. Ferrofluid Labeling | | | |
| 3.1 | Dilute | Add 670 μ L of PBS-HSA to the mixture | Add 2.0 mL of PBS-HSA to the mixture |
| 3.2 | Mix | Gently mix the vial of SA-FF by inversion | |
| 3.3 | Add SA-FF | Add 130 μ L SA-FF, gently mix (Volume 1.6 mL) | Add 400 μ L SA-FF, gently mix (Volume 4.8 mL) |
| 3.4 | Incubate | Incubate for 5 minutes at RT | |
| 4. Cell Selection | | | |
| 4.1 | Dilute | Add 2.4 mL PBS-HSA, gently mix | Add 7.2 mL PBS-HSA, gently mix |
| 4.2 | Separation | Insert tube into the quadropole for 10 minutes to allow cells labeled with SA-FF to be drawn to the walls of the tube | |
| 4.3 | Aspiration | Carefully aspirate the supernatant with a Pasteur Pipette without touching the tube's sides | |
| 4.4 | Resuspension | Remove the tube from the QP5, add 4.0 mL of PBS-HSA Gently mix the tube to resuspend | Remove the tube from the QP15, add 12.0 mL of PBS-HSA Gently mix the tube to resuspend |
| 4.5 | Wash cells (may be repeated) | Insert tube into the quadropole for 10 minutes Carefully aspirate the supernatant with a Pasteur Pipette without touching the tube's sides Either resuspend as per step 4.4 and repeat cell capture (4.2) or skip to 4.6 | |
| 4.6 | Resuspension | Remove from the quadropole and resuspend in PBS-HSA or desired medium | |

Precautions and Disclaimers:

This product is for Research Use Only, not for use in Diagnostic Procedures, and for *ex vivo* use only. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices. The kit should not be used post expiration dating. There may be excess material in the vials due to the product specific requirements for use.

This product is manufactured in the USA entirely from material of non-animal origin. The manufacture, packaging, storage, and transportation of these materials do not involve the use of material of animal origin. This information is to be used for the purpose of determining animal origin only and not to be confused with 'country of origin' for import/export purposes.

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Certifications:

BioMagnetic Solutions' Quality Management System is certified to ISO 9001:2015 and ISO 13485:2016 by NQA.