

Product Description:

This kit is intended for the positive selection of CD3⁺ 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 uses 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 CD3⁺ T cells from 2.0 – 4.0 x 10⁸ PBMC.

Product Contents	
Biotinylated anti-CD3 mAb	1 vial: 1.0 mL, 12 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 the CD3⁺ cell selection studies:

Phosphate Buffered Saline (minus Ca²⁺ and Mg²⁺) with 2.0 mM 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 or produced by the user.
 - Recommended PBS: Corning PBS (Cat. No: 21-0031-CV) or equivalent.
 - Recommended EDTA: Fisher Scientific (Cat: S311) or equivalent.
- Human Serum Albumin (HSA)
 - Recommended HSA (25%): Akron, (Cat. No: AK8228-0100) or equivalent.

Disposable Tubes 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 of reagents to cells

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 Quadrupole.

		QP5 Quadrupole Selection	QP15 Quadrupole Selection
Application:		Final volume of cells approximately 4.0 mL using a 12 x 75 mm tube	Final volume of cells approximately 12.0 mL using a 17 x 120 mm tube
1. Cell Preparation			
1.1	Wash Cells	Wash cells 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
1.3	<i>Optional: If sample contains monocytes, add Human IgG (H-IgG) to block non-specific binding.</i>	Add H-IgG to the cells to a final concentration of 1.0 mg/mL	
1.4		Add 80 μ L of recommended H-IgG (Volume approximately 0.48 mL)	Add 240 μ L of recommended H-IgG (Volume approximately 1.44 mL)
1.5		Incubate after gentle mixing for 5 min at RT	
2. Antibody Labeling			
2.1	Dilute	Add 120 μ L of PBS-HSA to cell mixture	Add 360 μ L of PBS-HSA to cell mixture
2.2	Mix	Gently mix the vial of anti-CD3 mAb	
2.3	Add mAb	Add 200 μ L mAb to the cells, gently mix (Volume approximately 0.80 mL)	Add 600 μ L mAb to the cells, gently mix (Volume approximately 2.4 mL)
2.4	Incubate	Incubate for 5 minutes at RT	
3. Ferrofluid Labeling			
3.1	Dilute	Add 600 μ L of PBS-HSA to the mixture	Add 1.8 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 220 μ L SA-FF, gently mix (Volume approximately 1.62 mL)	Add 660 μ L SA-FF, gently mix (Volume approximately 4.86 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 quadrupole 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 magnet, add 4.0 mL of PBS-HSA and gently resuspend the cells	Remove the tube from the QP15, add 12.0 mL of PBS-HSA and gently resuspend the cells
4.5	Wash cells (may be repeated)	Insert the tube containing the selected cell population into the quadrupole for 10 minutes Carefully aspirate the supernatant with a Pasteur Pipette without touching the tube's sides Resuspend in quadrupole volume (or skip to resuspension in 4.6)	
4.6	Resuspension	Remove from the quadrupole 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.