Product Insert: FerroSelect™ AbsenT™ CD4 Negative Selection Kit - QP (Cat. 28-0035)

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Product Description:

Negative selection of $CD4^+$ 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 $CD4^+$ T cells from approximately 2.4 – 4.8 x 10^8 PBMC.

Product Contents	
Biotinylated AbsenT™ mAb	1 vial: 1.0 mL, 12 µg/mL in PBS w/ 1.0% rHSA
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 the negative selection of $CD4^+$ cells:

Phosphate Buffered Saline containing 1.0% HSA (PBS-HSA)

- Phosphate buffered saline supplied either separately by BioMagnetic Solutions or produced by the user
  - Recommended PBS: Corning PBS (Cat. No: 21-0030-CV) or equivalent
  - NOTE: Do **NOT** use PBS free of $Ca^{2+}$and Mg^{2+}
- 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)

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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:		Separation of 0.8 – 1.6 x 10^8 PBMC using a 12 x 75 mm tube	Separation of 2.4 – 4.8 x 10^8 PBMC using a 17 x 120 mm tube
1. Cell Preparation			
1.1	*Optional: Wash Cells*	Wash cells by centrifugation in PBS-HSA. BioMagnetic Solutions data shows the product does not need to be washed prior to antibody labeling, but it may be washed if required	
1.2	Resuspend Cells	Resuspend to 2.0 – 4.0 × 10^8 cells/mL and aliquot 0.4 mL into a fresh tube	Resuspend to 2.0 – 4.0 × 10^8 cells/mL and aliquot 1.2 mL into a fresh tube
2. Antibody Labeling			
2.1	Dilute	Add 100 µL of PBS-HSA to cell mixture	Add 320 µL of PBS-HSA to cell mixture
2.2	Mix	Gently mix the vials of AbsenT mAb and anti-CD8 mAb	
2.3	Add mAbs	Add 140 µL AbsenT mAb to the cells Add 160 µL Anti-CD8 mAb to the cells Gently mix (Volume 0.80 mL)	Add 400 µL AbsenT mAb to the cells Add 480 µL Anti-CD8 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 480 µL of PBS-HSA to the mixture	Add 1.44 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 320 µL SA-FF, gently mix (Volume 1.6 mL)	Add 960 µL 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 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	Results	The collected supernatant is the fraction containing untouched $CD4^+$ T Cells	

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.

Limited Use Label License:

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Certifications:

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