Technical Data Sheet

Anti-Human CD45RO Particles - DM

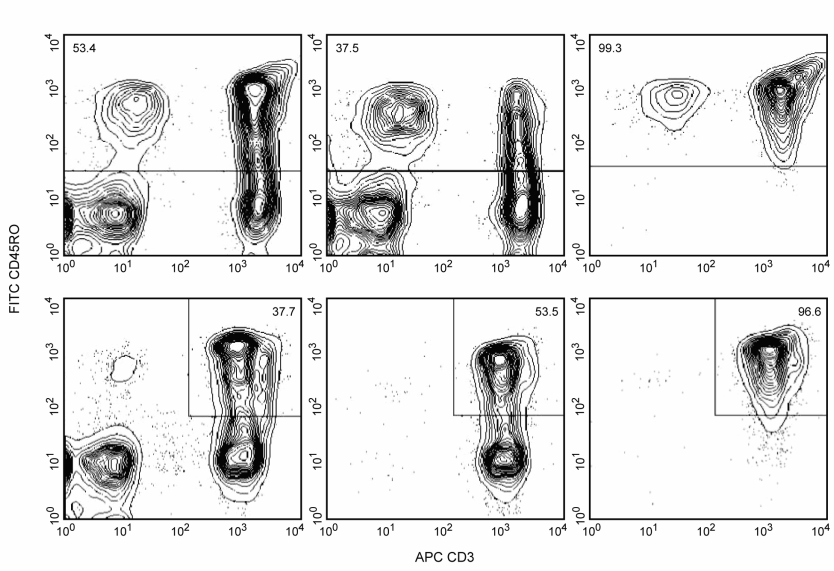
Product Information

Material Number:	**557986**
Size:	5.0 ml
Clone:	UCHL1
Isotype:	Mouse IgG2a κ
Reactivity:	QC Testing: Human
Workshop:	IV N31
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Anti-Human CD45RO Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection of CD45RO-bearing leukocytes using the BD IMagnet™ (Cat. No. 552311). CD45RO is the 180 kDa isoform of leukocyte common antigen that is not encoded by exon A, B, or C and found on most thymocytes, activated T lymphocytes, granulocytes, and monocytes and on a major subset of peripheral T lymphocytes. CD45RO and CD45RA expression defines complementary, predominantly non-overlapping populations of T cells in peripheral blood; and it is generally accepted that naive T cells are CD45RO- CD45RA+, while memory T cells are CD45RO+ CD45RA-. To specifically enrich CD45RO-expressing memory T lymphocytes, we recommend first depleting the erythrocytes, platelets, and non-T leukocytes, by using the appropriate BD IMag™ human T lymphocyte enrichment set, followed by positive selection of the CD45RO+ population (please refer to the following protocol).

This antibody is routinely tested by cell separation. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Positive selection of CD45RO+ leukocytes and CD45RO+ T lymphocytes from PBMC collected from two different donors. *Leukocytes from the first donor (top three panels) were labeled with BD™ IMag Anti-Human CD45RO Particles - DM as desribed in the Protocol. After labeling, the cells were separated using the BD™ IMagnet, and the negative (CD45RO-) and positive (CD45RO+) fractions were collected. T lymphocytes were enriched from the second donor's PBMC (bottom three panels) using the BD™ IMag Human T Lymphocyte EnrichmentSet (Cat. No. 557874) and then the CD45RO+ cells were collected. Please refer to the Positive Selection Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, fresh PBMC (top and bottom left panels), the negative fraction (top middle panel), the positive fraction (top right panel), enriched T lymphocytes (bottom middle panel), and CD45RO+ T lymphocytes (bottom right panel) were stained with FITC-conjugated anti-human CD45RO mAb UCHL1 (Cat. No. 555492) and APC-conjugated anti-human CD3 mAb UCHT1 (Cat. No. 555335). Non-viable cells were excluded from analysis by staining with propidium iodide. The percentages of CD45RO+ cells (top three panels) or CD45RO+ T lymphocytes (bottom three panels) in each sample is given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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Preparation and Storage

Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.
Store undiluted at 4° C.

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:
Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ Anti-Human CD45RO Particles - DM according to the following Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet™. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (negative fraction). The tube is then removed from the magnetic field for resuspension of the positive fraction. The separation is repeated twice to increase the purity of the positive fraction. The magnetic separation steps are diagrammed in the Separation Flow Chart. After the positive fraction is washed, it can be further evaluated in downstream applications such as flow cytometry and tissue culture.

MAGNETIC LABELING AND SEPARATION PROTOCOL
1. Dilute BD IMag™ Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide), and store at 4°C.

Optional: *If only CD45RO-positive T cells are desired, enrich the T lymphocytes by using the BD IMag™ Human T Lymphocyte, CD4 T Lymphocyte, or CD8 T Lymphocyte Enrichment Set - DM (Cat. No. 557874, 557939, or 557941, respectively).*

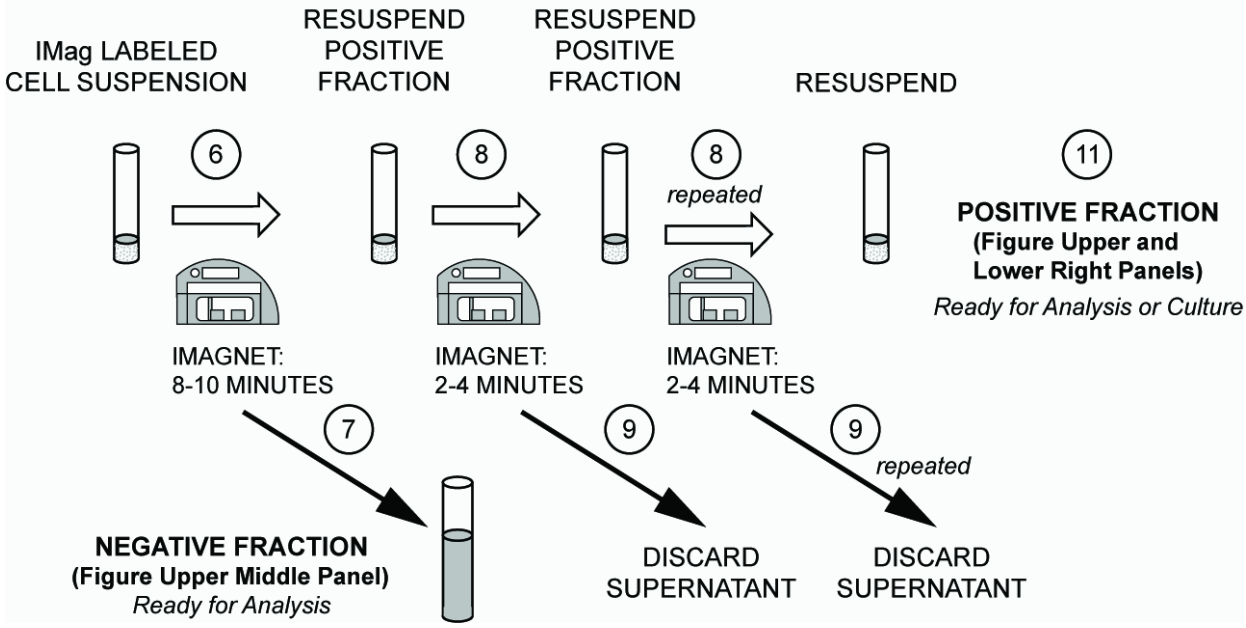
2. Prepare PBMC from anti-coagulated human blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
3. Count the cells, wash them with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ Anti-Human CD45RO Particles - DM thoroughly, and add 50 µl of particles for every 10^7 total cells.
5. **MIX THOROUGHLY.** Incubate at room temperature for 30 minutes.†
6. Bring the BD IMag™-particle labeling volume up to 1 - 8 x 10^7 cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™ (Cat. No. 552311). Incubate for 8 - 10 minutes.
7. With the tube on the BD IMagnet™, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
8. Remove the tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 6. Gently resuspend cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 - 4 minutes.
9. With the tube on the BD IMagnet™, carefully aspirate off the supernatant and discard.
10. Repeat Steps 8 and 9.
11. After the final wash step, resuspend the positive fraction in an appropriate buffer or medium, and proceed with desired downstream application(s).

NOTES:
* Hints for successful cell preparation:
-Draw the blood into a tube containing EDTA (for example, BD Vacutainer EDTA tube, Cat. No. 366457 or 367661).
-Remove the platelet rich plasma by centrifuging once at 220-240 × g.
-Wash 2-3 times in PBS after the density gradient separation.
-Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.

† Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.
Ficoll is a trademark of Amersham Biosciences AB
Paque is a trademark of Amersham Health AS

POSITIVE SELECTION FLOW CHART

(The circled numbers correspond to the steps of the following Protocol.)



Suggested Companion Products

Catalog Number	Name	Size	Clone
552362	BD IMag™ Buffer (10X)	100 ml	(none)
552311	Cell Separation Magnet	each	(none)
557874	BD IMag™ Human T Lymphocyte Enrichment Set-DM	each	(none)
555335	APC Mouse Anti-Human CD3	100 tests	UCHT1
555492	FITC Mouse Anti-Human CD45RO	100 tests	UCHL1

Product Notices

1. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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