Technical Data Sheet

Anti-Human CD45RA Particles - DM

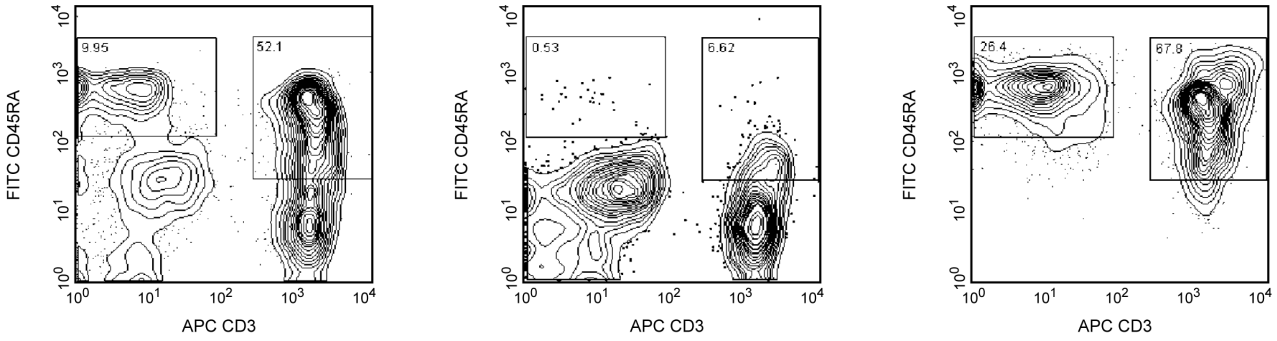
Product Information

Material Number:	**557981**
Size:	5.0 ml
Clone:	HI100
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Human
Workshop:	IV N906
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD™ IMag Anti-Human CD45RA Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of the CD45RA-bearing leukocytes using the BD™ IMagnet (Cat. No. 552311). CD45RA is the 220 kDa isoform of the human leukocyte common antigen found on major subsets of peripheral CD4+ and CD8+ T lymphocytes, B lymphocytes, NK cells, and monocytes. 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 CD45RA-expressing naive 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 CD45RA+ population (please refer to the following protocol).

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



Positive selection of human CD45RA+ Leukocytes from PBMC. *Leukocytes were labeled with BD™ IMag Anti-Human CD45RA Particles - DM as described in the protocol. After labeling, the cells were separated using the BD™ IMagnet, and the negative (CD45RA-) and positive (CD45RA+) fractions were collected. Please refer to the Separation Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, fresh PBMC (left panel), the negative fraction (middle panel) and the positive fraction (right panel) were stained with FITC-conjugated anti-human CD45RA mAb HI100 (Cat. No. 555488) 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 CD45RA+ CD3- and CD45RA+ CD3+ cells in each sample is given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

BD Biosciences

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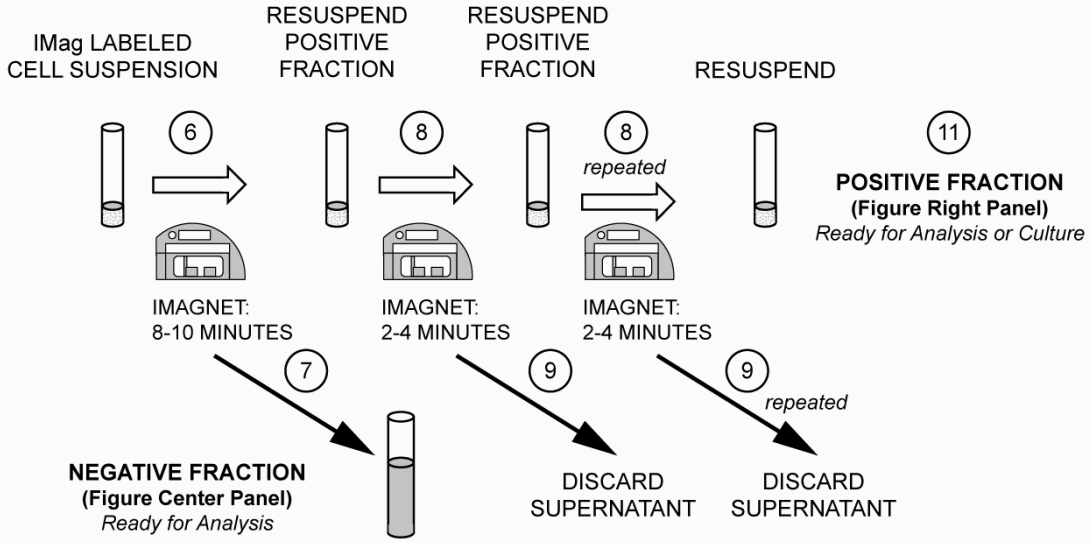
SEPARATION FLOW CHART

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



Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ Anti-Human CD45RA 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 CD45RA-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 CD45RA 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:
  - o Draw the blood into a tube containing EDTA (for example, BD Vacutainer EDTA tube, Cat. No. 366457 or 367661).
  - o Remove the platelet rich plasma by centrifuging once at 220-240 × g.
  - o Wash 2-3 times in PBS after the density gradient separation.
  - o 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.

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AB Paque is a trademark of Amersham Health AS

Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
555488	FITC Mouse Anti-Human CD45RA	100 tests	HI100
555335	APC Mouse Anti-Human CD3	100 tests	UCHT1
552362	Buffer (10X)	100 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
4. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
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

Johnson P, Maiti A. CD45: A family of leukocyte-specific cell surface glycoproteins. In: Herzenberg LA, Weir DM, Blackwell C, ed. *Weir's Handbook of Experimental Immunology, Vol 2.* Cambridge: Blackwell Science; 1997:62.1-62.16.(Biology)
Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LW. Control of lymphocyte recirculation in man. I. Differential regulation of the peripheral lymph node homing receptor L-selectin on T cells during the virgin to memory cell transition. *J Immunol.* 1993; 150(3):1105-1121.(Biology)
Schwinzer R. Cluster Report: CD45/CD45R. In: Knapp W, Dorken B, Rieber EP, et al, ed. *Leukocyte Typing IV: White Cell Differentiation Antigens.* New York: Oxford University Press; 1989:628-634.(Clone-specific)