Technical Data Sheet

Anti-Human CD3 Magnetic Particles - DM

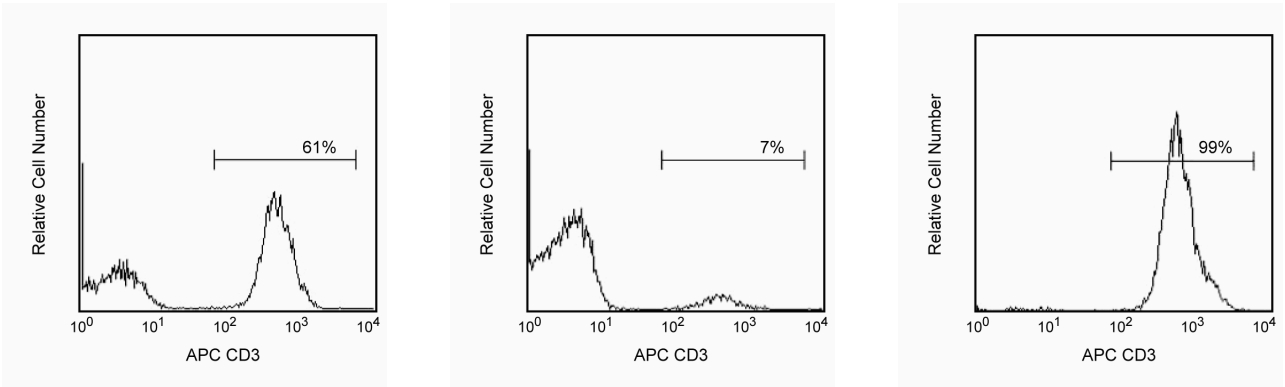
Product Information

Material Number:	**552593**
Size:	5.0 ml
Clone:	HIT3a
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Anti-Human CD3 Particles DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD3-bearing leukocytes using the BD IMagnet™. CD3 has been reported to be expressed on all mature T lymphocytes and on developing thymocytes.

Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ anti-human CD3 Particles - DM according to the Magnetic Labeling Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. No. 552311). 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 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, the small size of the magnetic particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.



Positive selection and depletion of human CD3-positive PBMC. *Leukocytes were labeled with BD IMag™ anti-human CD3 Particles - DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD3-) and positive (CD3+) 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 APC-conjugated anti-human CD3 mAb UCHT1 (Cat. No. 555335). The percent CD3+ cells in each sample is given.*

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

MAGNETIC LABELING PROTOCOL

1. Prepare PBMC from anti-coagulated human blood, preferably by density gradient centrifugation using Ficoll-Paque™. Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.
2. Dilute BD IMag™ Buffer (10X) (Cat. no. 552362) 1:10 with sterile distilled water or prepare 1X BD IMag™ buffer by supplementing Phosphate Buffered Saline with 0.5% BSA, 2 mM EDTA, and 0.09% sodium azide). Store at 4°C.
3. Wash cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ anti-human CD3 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.

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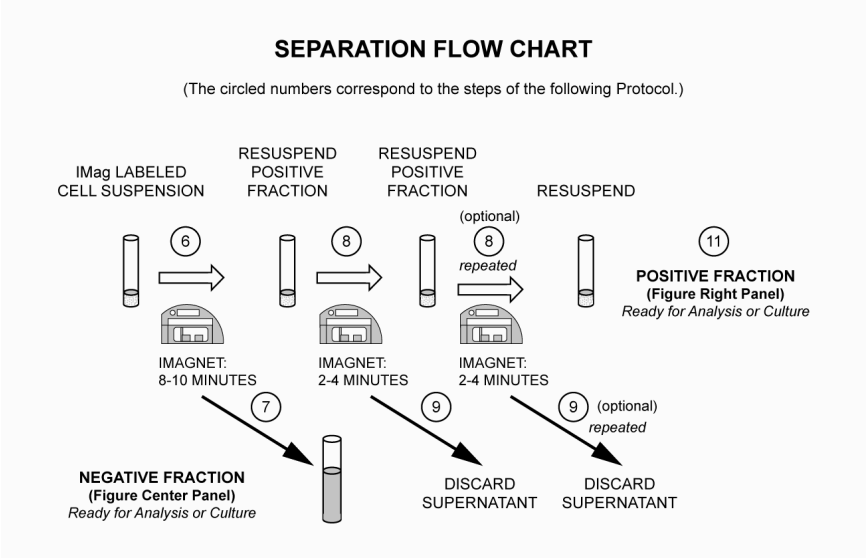
6. Bring the BD IMag-particle labeling volume up to 1 - 8 x 10^7 cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™. Incubate for 8 - 10 minutes.
7. With the tube on the BD IMagnet™, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
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.Typically, after one wash, the purity of the positive fraction will be greater than 98%. If greater purity is desired, Steps 8 and 9 may be repeated to obtain purities greater than 99.0%.
11.After the final wash step, resuspend the positive fraction in an appropriate buffer or media, and proceed with desired downstream application(s).

The concentration of the BD IMag™ anti-human CD3 Particles -DM has been optimized for the purification of CD3-positive T lymphocytes from human peripheral blood. When labeling target cell populations present at lower frequencies, fewer BD IMag™ particles can be used. Conversely, when labeling target cell populations that are present in higher frequencies, more particles should be used. To determine the optimal concentration of the BD IMag™ anti-human CD3 Particles -DM for a particular application, a titration in two-fold increments is recommended.

NOTE: Avoid nonspecific labeling by working quickly and keeping incubation times to a minimum.



Suggested Companion Products

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

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology and are licensed under US patent number 7,169,618.
3. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Knapp W, Dorken B, Rieber EP, et al, ed. *Leucocyte Typing IV.* New York: Oxford University Press; 1989:1-1208. (Biology)
McMichael AJ, Beverly PCL, Gilks W, et al, ed. *Leukocyte Typing III: White Cell Differentiation Antigens.* New York: Oxford University Press; 1987. (Biology)
Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens.* New York: Oxford University Press; 1995. (Biology)