Technical Data Sheet

Anti-Human CD14 Magnetic Particles - DM

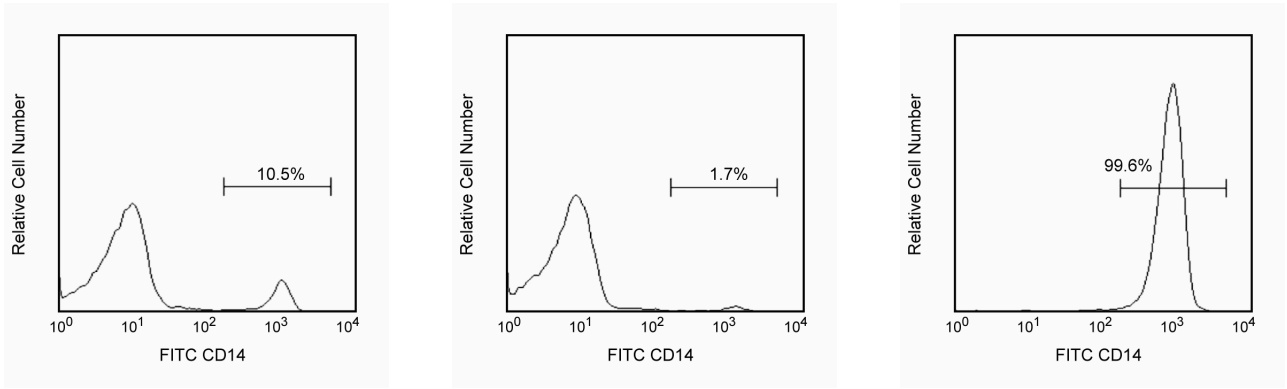
Product Information

Material Number:	**557769**
Size:	5.0 ml
Clone:	MΦP9
Reactivity:	QC Testing: Human Tested in development: Rhesus macaque
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Anti-Human CD14 Magnetic Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD14-bearing leukocytes using the BD IMagnet™. CD14 has been reported to be expressed at high levels on peripheral blood monocytes. Additionally, it is found on interfollicular macrophages, reticular dendritic cells, and some Langerhans cells. BD IMag™ Anti-Human CD14 magnetic particles have been reported to effectively separate the CD14-bearing cells of rhesus macaque blood.

Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ Anti-human CD14 Magnetic 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 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, 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 CD14+ PBMC. *Leukocytes were labeled with BD IMag™ Anti-Human CD14 Magnetic Particles - DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD14-) and positive (CD14+) 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 mouse anti-human CD14 antibody (clone M5E2) (Cat. No. 555397). The percent CD14+ cells in each sample is given.*

Preparation and Storage

Store undiluted at 4° C.

Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.

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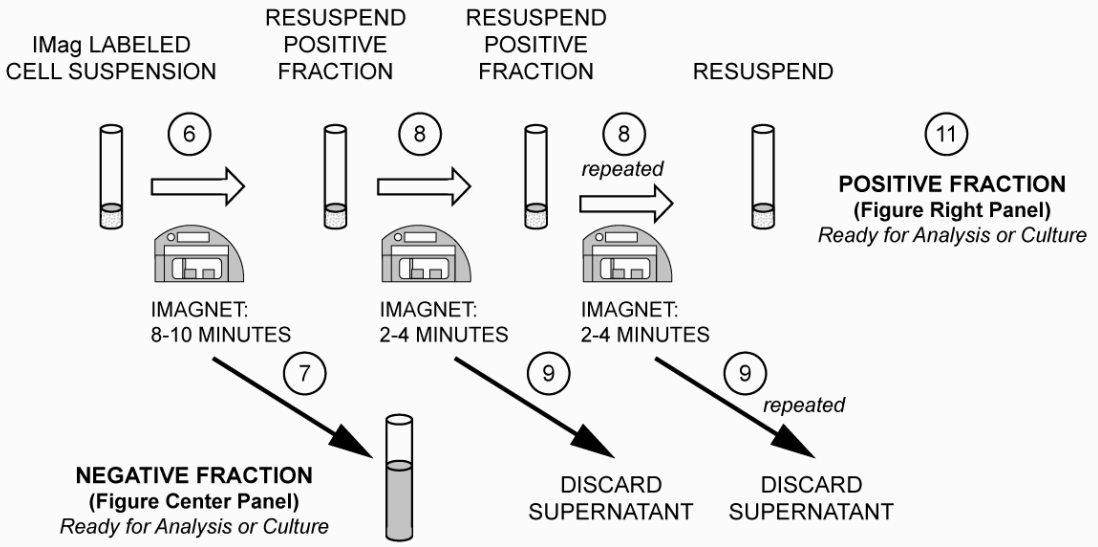
SEPARATION FLOW CHART

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



Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:
Magnetic Labeling Protocol

1. Prepare PBMC from anti-coagulated human (or rhesus macaque) blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
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. 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 CD14 Magnetic Particles - DM thoroughly, and add 50 µl of particles for every 10e7 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 10e7 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. Repeat Steps 8 and 9.
11. After the final wash step, resuspend the positive fraction in an appropriate buffer or media, and proceed with desired downstream application(s).

*Hints for successful cell preparation:

Draw the blood into a tube containing EDTA (e.g BD Vacutainer® collection tubes MN 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.

† The BD IMag™ particles may need to be titrated to optimize the separation of rhesus macaque leukocytes.
‡ Avoid nonspecific labeling by working quickly and adhering to the recommended incubation times.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
555397	FITC Mouse Anti-Human CD14	100 tests	M5E2
552362	Buffer (10X)	100 ml	(none)

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.
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

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

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