Datasheet for ABIN1305350

Anti-Human CD4 Magnetic Particles

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Overview

Quantity:	5 mL
Target:	CD4
Reactivity:	Human, Baboon, Cynomolgus, Rhesus Monkey
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	Magnetic Particles
Application:	Separation (Sep)

Product Details

Brand:	BD IMag™

Target Details

Target:	CD4
Alternative Name:	CD4 (CD4 Products)
Background:	BD IMag™ anti-human CD4 Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD4-bearing T lymphocytes using the BD IMagnet™. The L200 antibody reacts with CD4 on human, rhesus and cynomolgus macaque, and baboon peripheral blood leukocytes, we have confirmed that the BD IMag ™ particles can effectively separate the CD4-bearing cells of rhesus macaque blood. The distribution of CD4 on peripheral leukocytes is similar for both human and monkey. It is on the MHC class II-restricted T helper cells, with the majority of CD4-positive lymphocytes being CD8-negative. It is also found on most thymocytes

Target Details

and at low density on monocytes, it is not found on B or NK cells.

Application Details

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 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 CD4 Particles - DM thoroughly, and add 50 µl of particles for every 10^7 total cells. The BD IMag™ particles may need to be titrated to optimize the separation of rhesus macaque leukocytes.
5. MIX THOROUGHLY. Incubate at room temperature for 30 minutes. Avoid nonspecific labeling by working quickly and adhering to the recommended incubation times. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
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 1 ml of 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).

NOTES:

Hints for successful cell preparation:

a) Draw the blood into a tube containing EDTA (for example, BD Vacutainer EDTA tube, Cat. No. 366457 or 367661).

b) Remove the platelet rich plasma by centrifuging once at 220-240 x g.

c) Wash 2-3 times in PBS after the density gradient separation.

d) Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell

Application Details

	strainer.
Assay Procedure:	Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ anti-human CD4 Particles - DM according to the following Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. No. 552311). Labeled cells migrate toward the magnetic (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.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Aqueous buffered solution containing BSA and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed. Store undiluted at 4°C.

Publications

Product cited in:	Verdier, Aujoulat, Condevaux, Descotes: "Determination of lymphocyte subsets and cytokine levels in cynomolgus monkeys." in: **Toxicology**, Vol. 105, Issue 1, pp. 81-90, (1996) (PubMed). Wilson, Shooshtari, Finerty, Watkins, Morgan: "Selection of monoclonal antibodies for the identification of lymphocyte surface antigens in the New World primate Saguinus oedipus oedipus (cotton top tamarin)." in: **Journal of immunological methods**, Vol. 178, Issue 2, pp. 195-200, (1995) (PubMed). Jacobsen, Aasted, Broe, Petersen: "Reactivities of 20 anti-human monoclonal antibodies with

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leucocytes from ten different animal species." in: **Veterinary immunology and immunopathology**, Vol. 39, Issue 4, pp. 461-6, (1994) (PubMed).

Savary, Lotzová, Jackson, Jardine, Ang: "Analysis of interleukin-2-activated killer cells of rhesus monkeys: striking resemblance to the human system." in: **Journal of leukocyte biology**, Vol. 54, Issue 4, pp. 307-13, (1993) (PubMed).

Indzhiia, Yakovleva, Overbaugh, Licciardi, Chikobava, Klotz, Torres, Indzhiia, Lapin, Clark: " Baboon T cell lymphomas expressing the B cell-associated surface proteins CD40 and Bgp95." in: **Journal of clinical immunology**, Vol. 12, Issue 3, pp. 225-36, (1992) (PubMed).

There are more publications referencing this product on: Product page

Images

Flow Cytometry

Image 1.