Datasheet for ABIN1305355

Anti-Mouse CD8a Magnetic Particles

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Overview

Quantity:	10 mL
Target:	CD8 alpha (CD8A)
Reactivity:	Mouse
Host:	Rat
Clonality:	Monoclonal
Conjugate:	Magnetic Particles
Application:	Separation (Sep)

Product Details

Brand:	BD IMag™

Target Details

Target:	CD8 alpha (CD8A)
Alternative Name:	CD8a (CD8A Products)
Background:	BD IMag™ anti-mouse CD8a Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD8a-bearing leukocytes using the BD IMagnet™. CD8a has been reported to be expressed on most thymocytes and a subpopulation of mature T lymphocytes (e.g. MHC class I-restricted T cells, including most T suppressor/cytotoxic cells). In addition, subsets of gammadelta TCR-bearing T cells, intestinal intraepithelial lymphocytes, and dendritic cells also have been reported to express CD8a. Leukocytes are labeled with BD IMag™ anti-mouse CD8a Particles - DM according to the

Target Details

Magnetic Labeling Protocol. This labeled cell suspension is then placed within the magnetc field of 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 seperation 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.

Application Details

Protocol:

1. Prepare a single-cell suspension from the lymphoid tissue of interest according to standard laboratory procedures. 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. Place on ice. Although our experience indicates that the use of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) is not required for optimal cell separation, some laboratories may want to use it in their studies. If adding Mouse BD Fc Block, proceed to Step 3. If not adding Mouse BD Fc Block, proceed to Step 4.
3. Add Mouse BD Fc Block at 0.25 µg/10^6 cells, and incubate on ice for 15 minutes.
4. Wash cells with at least an equal volume of 1X BD IMag buffer, and carefully aspirate all the supernatant.
5. Vortex the BD™ IMag anti-mouse CD8a Particles - DM thoroughly, and add 50 µl of particles for every 10^7 total cells.
6. MIX THOROUGHLY. Refrigerate at 6°C - 12°C for 30 minutes.
7. 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 at room temperature for 6 - 8 minutes.
8. With the tube on the BD IMagnet™, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
9. Remove the tube from the BD IMagnet™, and add 1X BD IMag buffer to the same volume as in Step 7. Gently resuspend cells by pipetting briefly, and return the tube to the BD IMagnet™ for another 2 - 4 minutes.
10. With the tube on the BD IMagnet™, carefully aspirate off the supernatant and discard.

Application Details

	11. Repeat Steps 9 and 10. 12. After the final wash step, resuspend the positive fraction in an appropriate buffer and at an appropriate concentration for further analysis. NOTE: Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.
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:	Store undiluted at 4° C.

Publications

Product cited in:	Traver, Akashi, Manz, Merad, Miyamoto, Engleman, Weissman: "Development of CD8alpha-positive dendritic cells from a common myeloid progenitor." in: **Science (New York, N.Y.)**, Vol. 290, Issue 5499, pp. 2152-4, (2000) (PubMed). Sydora, Brossay, Hagenbaugh, Kronenberg, Cheroutre: "TAP-independent selection of CD8+ intestinal intraepithelial lymphocytes." in: **Journal of immunology (Baltimore, Md. : 1950)**, Vol. 156, Issue 11, pp. 4209-16, (1996) (PubMed). Wang, Klein: "Thymus-neuroendocrine interactions in extrathymic T cell development." in: **Science (New York, N.Y.)**, Vol. 265, Issue 5180, pp. 1860-2, (1994) (PubMed). Lefrançois: "Extrathymic differentiation of intraepithelial lymphocytes: generation of a separate and unequal T-cell repertoire?" in: **Immunology today**, Vol. 12, Issue 12, pp. 436-8, (1992) (PubMed). Vremec, Zorbas, Scollay, Saunders, Ardavin, Wu, Shortman: "The surface phenotype of dendritic

cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells." in: **The Journal of experimental medicine**, Vol. 176, Issue 1, pp. 47-58, (1992) (PubMed).

There are more publications referencing this product on: Product page

Images

Flow Cytometry

Image 1.