Datasheet for ABIN1305234

Anti-Mouse CD117 Magnetic Particles

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Overview

Quantity:	5 mL
Target:	KIT
Reactivity:	Mouse
Host:	Rat
Clonality:	Monoclonal
Conjugate:	Magnetic Particles
Application:	Separation (Sep)

Product Details

Brand:	BD IMag™

Target Details

Target:	KIT
Alternative Name:	CD117 (KIT Products)
Background:	BD IMag™ anti-mouse CD117 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 CD117-bearing cells using the BD IMagnet™. The 2B8 antibody reacts with CD117 (c-Kit), a transmembrane tyrosine-kinase receptor which is encoded by the Kit gene (formerly dominant white spotting, W). The c-Kit ligand (also known as steel factor, stem cell factor, and mast cell growth factor) encoded by the Kit1 gene (formerly steel, Sl), is a co-mitogen for hematopoietic stem cells, myeloerythroid progenitors and a mast-cell differentiation factor. The KitW and Kit1Sl mutant alleles have similar pleiotropic effects on the

Target Details

development of melanocytes, germ cells, and the hematopoietic system. In the adult bone marrow, CD117 is expressed on hematopoietic progenitor cells, including CD90 (Thy-1) low, TER-119-, CD45R/B220-, CD11b (Mac-1)-, Ly-6G (Gr-1)-, CD4-, CD8-, and Sca-1 (Ly-6A/E)+ multipotent hemotopoietic stem cells, progenitors committed to myeliod and/or erythroid lineages, and precursors of B and T lymphocytes. This widespread expression of CD117 in hematopoietic precursors is consistent with the participation of c-Kit and its ligand in the regulation of several hematopoietic lineages. Intrathymic expression of c-Kit and c-Kit ligand suggest that CD117 is also involved in the regulation of some events during the development of T lymphocytes. CD117 is also expressed by mast cells and by dendritic cells found in the periarteriolar lymphocytoc sheaths (T-cell areas) of splenic white pulp. The mAb 2B8 reportedly does not block the action of c-Kit.

A single-cell suspension from the lymphoid tissue of interest is labeled with BD IMag™ anti-mouse CD117 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.

Synonyms: c-Kit

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-mm 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-mouse CD117 Magnetic Particles - DM thoroughly, and add 50 ml of particles for every 10 million total cells.

Application Details

	5. MIX THOROUGHLY. Refrigerate at 6°C to 12°C for 30 minutes. 6. Bring the BD IMag™-particle labeling volume up to 10 to 80 million cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™. Incubate at room temperature for 8-10 minutes. 7. With the tube on the BD IMagnet™, carefully pipette 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. 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 pipette 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). NOTE: The concentration of BD IMag™ anti-mouse CD117 Particles - DM suggested in the protocol has been optimized for the purification of CD117-positive cells from mouse bone marrow. 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 at higher frequencies, more particles should be used. To determine the optimal concentration of the BD IMag™ anti-mouse CD117 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 as recommended.
Restrictions:	For Research Use only

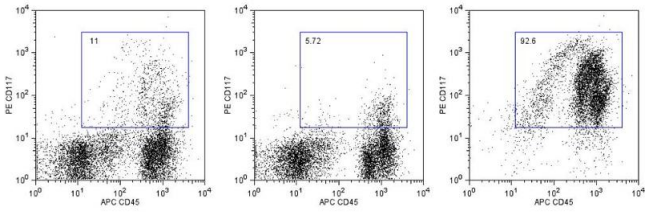
Handling

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 and protected from prolonged exposure to light. Do not freeze.

Publications

Product cited in:	Lian, Toki, Yu, Hayashi, Yasumizu, Sugiura, Jin, Inaba, Hisha, Li, Yu, Fan, Ikehara: "Intrathymically injected hemopoietic stem cells can differentiate into all lineage cells in the thymus: differences between c-kit+ cells and c-kit < low cells." in: **Stem cells (Dayton, Ohio)**, Vol. 15, Issue 6, pp. 430-6, (1998) (PubMed).

Images



Flow Cytometry

Image 1.