Technical Data Sheet

Anti-Mouse CD117 Magnetic Particles - DM

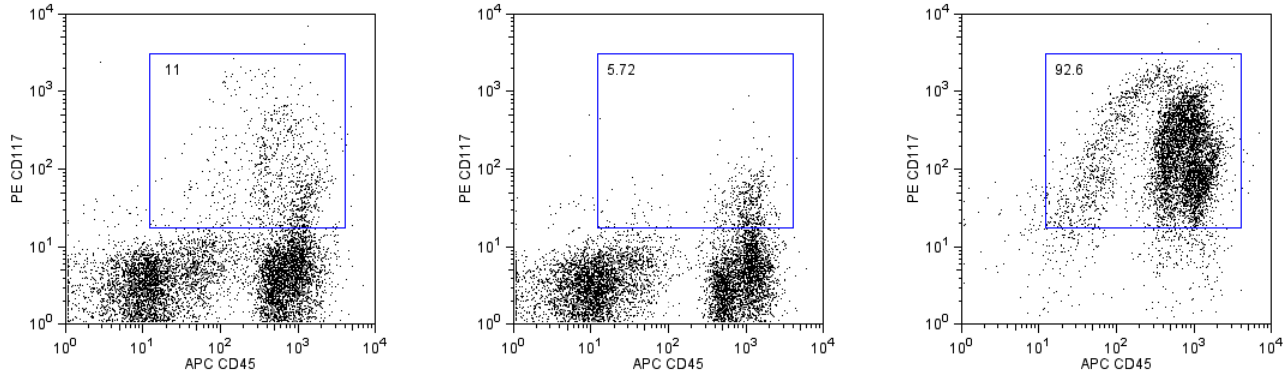
Product Information

Material Number:	**558620**
Alternate Name:	c-Kit
Size:	5.0 ml
Clone:	2B8
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

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, SI), is a co-mitogen for hematopoietic stem cells, myeloerythroid progenitors and a mast-cell differentiation factor. The KitW and Kit1SI mutant alleles have similar pleiotropic effects on the 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.



Positive selection of mouse CD117+ progenitor cells from mouse bone marrow. *Mouse bone marrow cells were labeled with BD IMag™ anti-mouse CD117 Particles - DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD117-) and positive (CD117+) 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 bone marrow cells (left panel), the negative fraction (middle panel), and the positive fraction (right panel) were stained with PE conjugated mouse anti-mouse CD117 mAb ACK45 (Cat. no. 553869) and APC-conjugated rat anti-mouse CD45 mAb 30-F11 (Cat. no. 559864). The percent CD117+/CD45+ cells in each sample is given.*

BD Biosciences

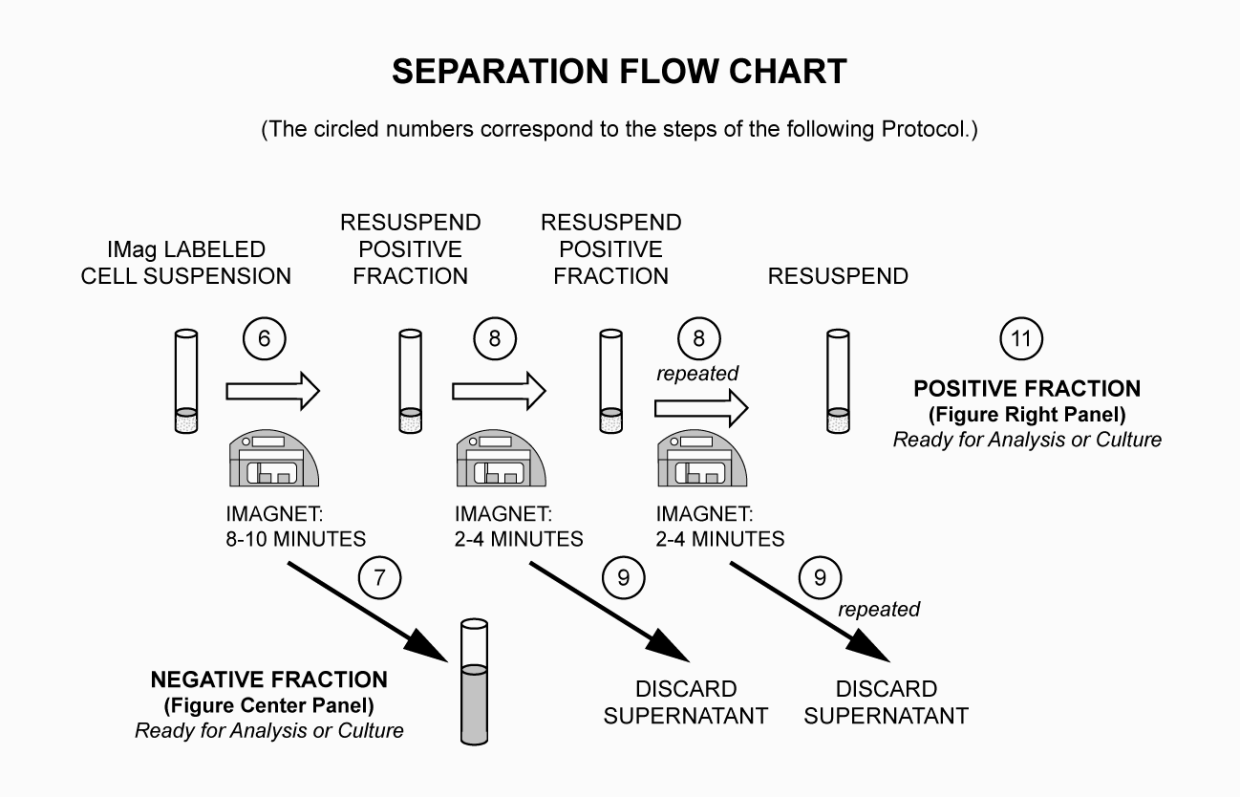
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.



Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

Magnetic Labeling 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 µl of particles for every 10 million total cells.
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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
552362	Buffer (10X)	100 ml	(none)
559864	APC Rat Anti-Mouse CD45	0.1 mg	30-F11

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. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Anderson DM, Lyman SD, Baird A, et al. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell.* 1990; 63(1):235-243. (Biology)

Austen KF, Boyce JA. Mast cell lineage development and phenotypic regulation. *Leuk Res.* 2001; 25(7):511-518. (Biology)

Domen J, Weissman IL. Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, Kitl/c-Kit signaling the other. *J Exp Med.* 2000; 192(12):1707-1718. (Biology)

Godfrey DI, Zlotnik A. Control points in early T-cell development. *Immunol Today.* 1993; 14(11):547-553. (Biology)

Huang E, Nocka K, Beier DR, et al. The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell.* 1990; 63(1):225-233. (Biology)

Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc Natl Acad Sci U S A.* 1992; 89(4):1502-1506. (Biology)

Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell.* 1997; 91(5):661-672. (Biology)

Lian Z, Toki J, Yu C, et al. Intrathymically injected hemopoietic stem cells can differentiate into all lineage cells in the thymus: differences between c-kit+ cells and c-kit < low cells. *Stem Cells.* 1997; 15(6):430-436. (Biology)

Mirmonsef P, Shelburne CP, Fitzhugh Yeatman C 2nd, Chong HJ, Ryan JJ. Inhibition of Kit expression by IL-4 and IL-10 in murine mast cells: role of STAT6 and phosphatidylinositol 3'-kinase. *J Immunol.* 1999; 163(5):2530-2539. (Biology)

Ogawa M, Matsuzaki Y, Nishikawa S, et al. Expression and function of c-kit in hemopoietic progenitor cells. *J Exp Med.* 1991; 174(1):63-71. (Biology)

Ogawa M, Nishikawa S, Yoshinaga K, et al. Expression and function of c-Kit in fetal hemopoietic progenitor cells: transition from the early c-Kit-independent to the late c-Kit-dependent wave of hemopoiesis in the murine embryo. *Development.* 1993; 117(3):1089-1098. (Biology)

Pulendran B, Lingappa J, Kennedy MK, et al. Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. *J Immunol.* 1997; 159(5):2222-2231. (Biology)

Rodewald HR, Kretzschmar K, Swat W, Takeda S. Intrathymically expressed c-kit ligand (stem cell factor) is a major factor driving expansion of very immature thymocytes in vivo. *Immunity.* 1995; 3(3):313-319. (Biology)

Valent P. The riddle of the mast cell: kit(CD117)-ligand as the missing link?. *Immunol Today.* 1994; 15(3):111-114. (Biology)

Zsebo KM, Wypych J, McNiece IK, et al. Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver--conditioned medium. *Cell.* 1990; 63(1):195-201. (Biology)