Technical Data Sheet

PE-Cy™5 Rat Anti-Mouse CD44

Product Information

Material Number:	**553135**
Alternate Name:	Pgp-1, H-CAM, Ly-24
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	IM7
Immunogen:	Dexamethasone-induced cells of the SJL mouse spontaneous myeloid leukemia M1
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The IM7 antibody reacts with an epitope on both alloantigens and all isoforms of the CD44 glycoprotein (Pgp-1, Ly-24). The standard form of CD44, lacking variable exons and referred to as CD44H or CD44s, is widely expressed on hematopoietic and non-hematopoietic cells. CD44 isoforms encoded by variable exons are expressed on epithelial cells, but only at low levels on most leukocytes. Mice with the Ly-24.1 alloantigen (e.g., BALB/c, CBA/J, DBA/1, DBA/2) have relatively large subsets of CD44H+ T lymphocytes, while Ly-24.2 strains (e.g., A, AKR, CBA/N, C3H/He, C57BL, C57BR, C57L, C58, NZB, SJL, SWR, 129) have few CD44H+ T cells. CD44 is a cell adhesion receptor, and its principal ligand, hyaluronate, is a common component of extracellular matrices. Differential glycosylation of CD44 influences its binding to hyaluronate. Additional ligands include the cell-surface form of CD74 and the cytokine osteopontin (Eta-1). Bone marrow- and thymus-derived progenitor cells capable of repopulating the thymus express CD44. In the periphery, the level of CD44 expression increases upon activation of B lymphocytes, CD4+ T cells, and CD8+ T cells; memory cells can be recognized by their CD44[hi] phenotype. The IM7 mAb inhibits established collagen-induced arthritis in DBA/1 mice. Moreover, it prevents CNS inflammation and clinical symptoms of experimental autoimmune encephalomyelitis. In contrast, the same antibody exacerbates experimental autoimmune thyroiditis in CBA/J mice. The IM7 mAb recognizes a different epitope from that recognized by mAb KM114 (Cat. No. 558739), and the antibody pair can be used in ELISA to detect soluble CD44. It has been observed that IM7 antibody cross-reacts with human, dog, cat, horse, cow, and pig leukocytes. Anti-human CD44, clone G44-26 (Cat. No. 555476), and IM7 antibody compete for binding to human peripheral blood lymphocytes.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553990	PE-Cy™5 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.
5. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.

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6. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
7. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
8. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
9. 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.
10. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™ (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.

References

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