

Technical Data Sheet

FITC Mouse Anti-Mouse Ly-49C and Ly-49I

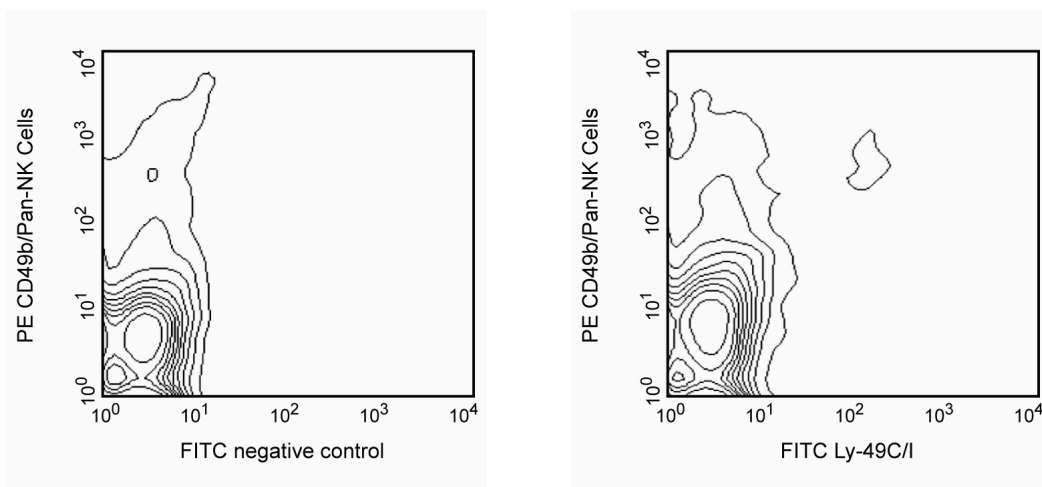
Product Information

Material Number:	562055
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	5E6
Immunogen:	Activated mouse NK cells
Isotype:	Mouse (129) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The 5E6 (also known as clone SW5E6) antibody reacts with Ly-49C[BALB], Ly-49C[B6], Ly-49C[NZB], and Ly-49I[B6], inhibitory receptors which are expressed on subsets of natural killer (NK) cells and NK-1.1+ (or DX5+) T lymphocytes (NK-T cells) in all strains tested except C57BR and RIII, on a population of memory CD8+ T lymphocytes and NK1.1+ $\gamma\delta$ T cells in C57BL/6 mice, and on a distinct subset of B-1 cells of BALB/c and C57BL/6 mice. The proportion of NK T cells expressing Ly-49C/I is higher (2-5 fold) in thymus than in liver (immature and mature NK T cells, respectively), and there is evidence that the down-regulation of Ly-49 receptor expression is necessary for normal NK T-cell development. Most NK cells express a single allele of Ly-49C, although occasionally they may express more than one allele. The Ly-49 family of NK-cell receptors are disulfide-linked type-II transmembrane protein homodimers with extracellular carbohydrate-recognition domains (CRD) that bind to MHC class I alloantigens. The Ly-49 family members are expressed independently, such that an individual NK or T cell may display more than one class of Ly-49 receptor homodimers. The 5E6 antibody is specific for the Ly-49C CRD. The Ly-49C[BALB] and Ly-49C[B6] alloantigens bind to MHC class I antigens of the b, d, k, and s haplotypes, and the 5E6 antibody blocks this binding. Binding of Ly-49C[BALB]- and Ly-49C[B6]- expressing transfectants to lymphoblasts of H-2[f], H-2[q], H-2[r], and H-2[v] strains has also been detected. Ly-49I[B6] transfectants bind H-2[r] lymphoblasts and bind much more weakly to the b, d, k, q, s, and v haplotypes. The levels of the Ly-49 inhibitory receptors are down-regulated by their ligands *in vivo*, and the various levels of expression of an Ly-49 inhibitory receptor may affect the specificity of NK cells. Ly-49C is specifically downregulated in the presence of H-2K[b] class I molecules (one of the Ly-49C ligands). Ly-49C[+] and/or Ly-49I[+] cells mediate allogeneic and hybrid resistance to H-2d bone marrow transplantation. *In vitro* and *in vivo* studies suggest that the Ly-49C and/or Ly-49I receptors mediate negative regulation of NK-cell cytolytic activity *via* tyrosine phosphorylation of their ITIMs (Immunoreceptor Tyrosine-based Inhibitory Motifs).

The epitope recognized by this antibody on Ly49C may be masked on freshly isolated primary NK cells due to cis interactions with MHC class I molecules. This observation has been reported for other Ly49C monoclonal antibodies that bind to the same structural region.



Two-color analysis of the expression of Ly-49C/I on splenic NK cells. C57BL/6 splenocytes were simultaneously stained with PE Rat anti-Mouse CD49b/Pan-NK cells mAb DX5 and FITC Mouse anti-Mouse Ly-49C and Ly-49I (right panel). Flow cytometry was performed on a BD FACScan™ flow cytometry system.

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Preparation and Storage

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

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
553456	FITC Mouse IgG2a, κ Isotype Control	0.25 mg	G155-178
553858	PE Rat Anti-Mouse CD49b	0.2 mg	DX5
554656	Stain Buffer (FBS)	500 ml	(none)

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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

References

Brennan J, Lemieux S, Freeman JD, Mager DL, Takei F. Heterogeneity among Ly-49C natural killer (NK) cells: characterization of highly related receptors with differing functions and expression patterns. *J Exp Med.* 1996; 184(6):2085-2090. (Biology)

Brennan J, Mahon G, Mager DL, Jefferies WA, Takei F. Recognition of class I major histocompatibility complex molecules by Ly-49: specificities and domain interactions. *J Exp Med.* 1996; 183(4):1553-1559. (Biology)

Coles MC, McMahon CW, Takizawa H, Raulet DH. Memory CD8 T lymphocytes express inhibitory MHC-specific Ly49 receptors. *Eur J Immunol.* 2000; 30(1):236-244. (Biology)

Hanke T, Takizawa H, McMahon CW, et al. Direct assessment of MHC class I binding by seven Ly49 inhibitory NK cell receptors. *Immunity.* 1999; 11(1):67-77. (Biology)

Hara T, Nishimura H, Hasegawa Y, Yoshikai Y. Thymus-dependent modulation of Ly49 inhibitory receptor expression on NK1.1+gamma/delta T cells. *Immunology.* 2001; 102(1):24-30. (Biology)

Held W, Kunz B. An allele-specific, stochastic gene expression process controls the expression of multiple Ly49 family genes and generates a diverse, MHC-specific NK cell receptor repertoire. *Eur J Immunol.* 1998; 28(8):2407-2416. (Biology)

Hoglund P, Sundback J, Olsson-Alheim MY, et al. Host MHC class I gene control of NK-cell specificity in the mouse. *Immunol Rev.* 1997; 155:11-28. (Biology)

Liu J, Yu YY, Lindahl KF, Kumar V, Bennett M. Allorecognition by murine natural killer cells: studies with bone marrow transplants and lysis of T lymphoblasts. *Chem Immunol.* 1996; 64:164-180. (Biology)

Mason LH, Gosselin P, Anderson SK, Fogler WE, Ortaldo JR, McVicar DW. Differential tyrosine phosphorylation of inhibitory versus activating Ly-49 receptor proteins and their recruitment of SHP-1 phosphatase. *J Immunol.* 1997; 159(9):4187-4196. (Biology)

Ochi H, Watanabe T. Negative regulation of B cell receptor-mediated signaling in B-1 cells through CD5 and Ly49 co-receptors via Lyn kinase activity. *Int Immunol.* 2000; 12(10):1417-1423. (Biology)

Olsson-Alheim MY, Salcedo M, Ljunggren HG, Karre K, Sentman CL. NK cell receptor calibration: effects of MHC class I induction on killing by Ly49Ahigh and Ly49ALow NK cells. *J Immunol.* 1997; 159(7):3189-3194. (Biology)

Ortaldo JR, Winkler-Pickett R, Mason AT, Mason LH. The Ly-49 family: regulation of cytotoxicity and cytokine production in murine CD3+ cells. *J Immunol.* 1998; 160(1):1158-1165. (Biology)

Raulet DH, Held W, Correa I, Dorfman JR, Wu MF, Corral L. Specificity, tolerance and developmental regulation of natural killer cells defined by expression of class I-specific Ly49 receptors. *Immunol Rev.* 1997; 155:41-52. (Biology)

Raziuddin A, Longo DL, Mason L, Ortaldo JR, Bennett M, Murphy WJ. Differential effects of the rejection of bone marrow allografts by the depletion of activating versus inhibiting Ly-49 natural killer cell subsets. *J Immunol.* 1998; 160(1):87-94. (Biology)

Raziuddin A, Longo DL, Mason L, Ortaldo JR, Murphy WJ. Ly-49 G2+ NK cells are responsible for mediating the rejection of H-2b bone marrow allografts in mice. *Int Immunol.* 1996; 8(12):1833-1839. (Biology)

Robson MacDonald H, Lees RK, Held W. Developmentally regulated extinction of Ly-49 receptor expression permits maturation and selection of NK1.1+ T cells. *J Exp Med.* 1998; 187(12):2109-2114. (Biology)

Salcedo M, Andersson M, Lemieux S, Van Kaer L, Chambers BJ, Ljunggren HG. Fine tuning of natural killer cell specificity and maintenance of self tolerance in MHC class I-deficient mice. *Eur J Immunol.* 1998; 28(4):1315-1321. (Biology)

Scarpellino L, Oeschger F, Guillaume P, Coudert JD, Lévy F, Leclercq G, Held W.. Interactions of Ly49 family receptors with MHC class I ligands in trans and cis.. *J Immunol.* 2007; 178(3):1277-1284. (Biology)

Sentman CL, Hackett J, Kumar V, Bennett M. Identification of a subset of murine natural killer cells that mediates rejection of Hh-1d but not Hh-1b bone marrow grafts. *J Exp Med.* 1989; 170(1):191-202. (Immunogen)

Skold M, Cardell S. Differential regulation of Ly49 expression on CD4+ and CD4-CD8- (double negative) NK1.1+ T cells. *Eur J Immunol.* 2000; 30(9):2488-2496. (Biology)

Stoneman ER, Bennett M, An J, et al. Cloning and characterization of 5E6(Ly-49C), a receptor molecule expressed on a subset of murine natural killer cells. *J Exp Med.* 1995; 182(2):305-313. (Biology)

Takei F, Brennan J, Mager DL. The Ly-49 family: genes, proteins and recognition of class I MHC. *Immunol Rev.* 1997; 155:67-77. (Biology)

Yu YY, George T, Dorfman JR, Roland J, Kumar V, Bennett M. The role of Ly49A and 5E6(Ly49C) molecules in hybrid resistance mediated by murine natural killer cells against normal T cell blasts. *Immunity.* 1996; 4(1):67-76. (Biology)

Yu YY, Kumar V, Bennett M. Murine natural killer cells and marrow graft rejection. *Annu Rev Immunol.* 1992; 10:189-213. (Biology)