

## Technical Data Sheet

## Biotin Mouse Anti-Mouse Ly-49C and Ly-49I

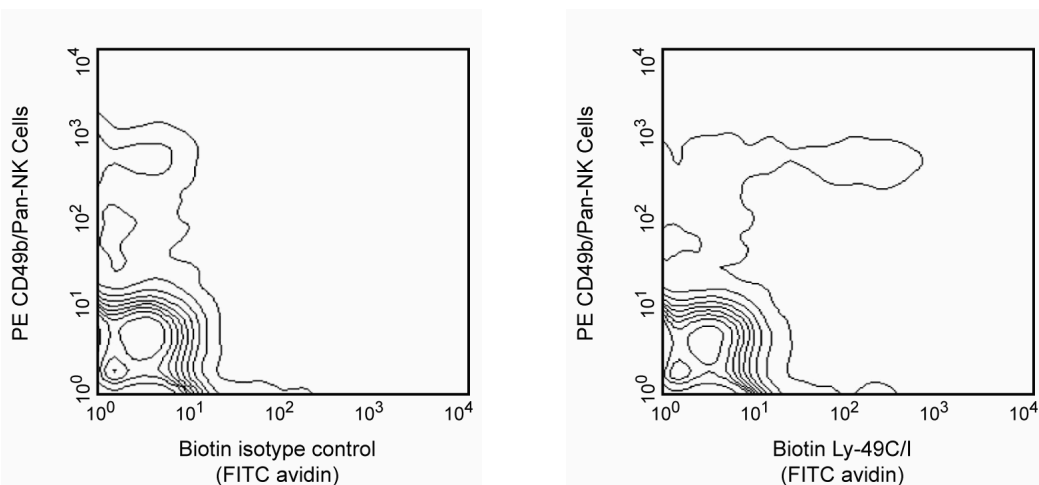
## Product Information

<b>Material Number:</b>	557418
<b>Alternate Name:</b>	Ly49C; Ly49I
<b>Size:</b>	0.1 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	5E6
<b>Immunogen:</b>	Activated mouse NK cells
<b>Isotype:</b>	Mouse (129) IgG2a, $\kappa$
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	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 (Cat. No. 553858) and either Biotin Mouse IgG2a,  $\kappa$  Isotype Control (Cat. No. 553455, left panel) or Biotin Mouse Anti-Mouse Ly-49C and Ly-49I (Cat. No. 557418, right panel), followed by Avidin FITC (Cat. No. 554057). Fluorescence contour plots were derived from gated events with the forward and side light-scattering characteristics of viable splenocytes. Flow cytometry was performed on a BD FACScan™.

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## Preparation and Storage

Store undiluted at 4°C.

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

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

## Application Notes

### Application

Flow cytometry	Routinely Tested
Immunoprecipitation	Reported

## Suggested Companion Products

Catalog Number	Name	Size	Clone
553858	PE Rat Anti-Mouse CD49b	0.2 mg	DX5
553455	Biotin Mouse IgG2a, $\kappa$ Isotype Control	0.25 mg	G155-178
554057	Avidin FITC	0.5 mg	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
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 Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

## References

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