

## Technical Data Sheet

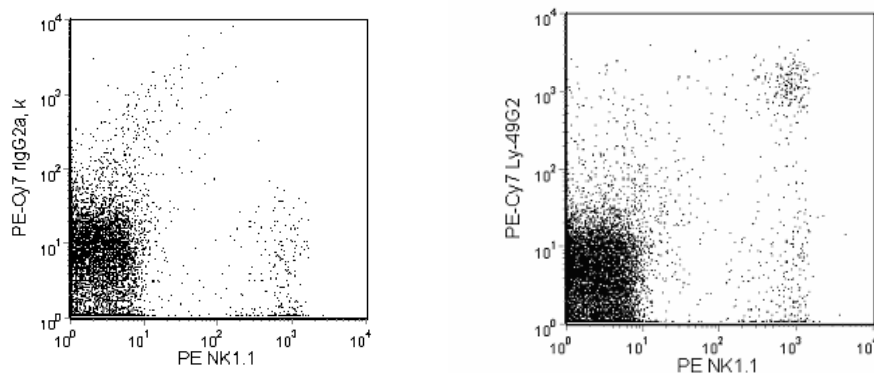
## PE-Cy™7 Rat Anti-Mouse Ly-49G2

## Product Information

Material Number:	560730
Alternate Name:	LGL-1
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	4D11
Immunogen:	Large granular lymphocytes (LGL) enriched from C57BL/6N mouse liver
Isotype:	Rat (F344) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

## Description

The 4D11 antibody reacts with Ly-49G2 (also known as LGL-1), an inhibitory receptor which is expressed on subsets of natural killer (NK) cells and DX5-positive T lymphocytes (NK-T cells) in all strains tested (e.g., AKR/N, BALB/c, C3H/HeJ, C57BL/6, CBA/J, DBA/2, SJL, 129) and on a population of memory CD8+ T lymphocytes in C57BL/6 mice. Cross-reaction of 4D11 antibody to Ly-49A[B6], Ly-49A[BALB], and Ly-49T[129/J] inhibitory receptors and Ly-49L[CBA/J] activating receptor has been reported. The proportion of NK-T cells expressing Ly-49A and Ly-49G2 is higher (2-5 fold) in thymus than in liver (immature and mature NK-T cells, respectively), and there is evidence that down-regulation of Ly-49 receptor expression is necessary for normal NK-T-cell development to occur. Most NK cells express a single allele of Ly-49A and/or Ly-49G2, although occasionally they may express more than one allele. The Ly-49 family of NK-cell receptors, members of the C-type lectin superfamily, are disulfide-linked type-II transmembrane protein homodimers with extracellular carbohydrate-recognition domains, which 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. Binding of Ly-49G[B6]-expressing transfectants to H-2Dd+/H-2Ld+ ConA blasts has been demonstrated, and H-2D[d]-expressing target cells inhibit the lytic activity of Ly-49G2-expressing NK cells. The levels of the Ly-49 inhibitory receptors are down-regulated by their ligands *in vivo*, and the various levels of expression of a Ly-49 inhibitory receptor may affect the specificity of NK cells. Ly-49G2[+] NK cells are able to lyse target tumor cells expressing H-2[a] and H-2[b] MHC class I antigens *in vitro*, and they mediate allogeneic and hybrid resistance to H-2[b] bone marrow transplantation. The Ly-49A[BALB] and Ly-49A[B6] alloantigens bind to MHC class I antigens of the d and k haplotypes, and Ly-49A[+] IL-2-activated NK cells are unable to lyse target cells expressing H-2[d] and H-2[k]. *In vitro* studies suggest that the Ly-49G2 and Ly-49A receptors mediate negative regulation of NK-cell cytolytic activity via tyrosine phosphorylation of their ITIMs (Immunoreceptor Tyrosine-based Inhibitory Motifs). Ly-49T[129/J] has a unique ITIM sequence, and Ly-49T-transfected 293T (human kidney epithelial) cells do not bind soluble tetramers of any tested H-2 alloantigen (D[b], D[d], D[k], K[b], K[d], K[k], L[d]).



**Flow cytometric analysis of Ly-49G2 on mouse splenocytes.** Splenocytes from C57BL/6 mice were stained with a PE Mouse Anti-Mouse NK1.1 antibody (Cat. No. 553165) in conjunction with either the PE-Cy™7 Rat Anti-Mouse Ly-49G2 antibody (right panel) or with a PE-Cy™7 Rat IgG2a, κ isotype control (left panel). Dot plots were derived from gated events based on light scattering characteristics for splenocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

## 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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

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## Application Notes

### Application

Flow cytometry

Routinely Tested

### Suggested Companion Products

Catalog Number	Name	Size	Clone
552784	PE-Cy <sup>TM</sup> 7 Rat IgG2a, $\kappa$ Isotype Control	0.1 mg	R35-95
553165	PE Mouse Anti-Mouse NK-1.1	0.2 mg	PK136
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block <sup>TM</sup> )	0.1 mg	2.4G2

### 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. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD<sup>TM</sup> Stabilizing Fixative (Cat. No. 338036).
4. 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.
5. 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.
6. 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.
7. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [wwwbdbiosciences.com/colors](http://wwwbdbiosciences.com/colors).
10. Please refer to [wwwbdbiosciences.com/pharming/protocols](http://wwwbdbiosciences.com/pharming/protocols) for technical protocols.

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