Technical Data Sheet

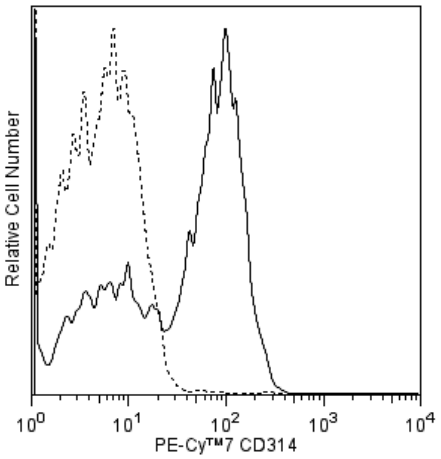
PE-Cy™7 Mouse Anti-Human CD314/NKG2D

Product Information

Material Number:	**562365**
Alternate Name:	CD314; KLRK1; KLR; NKG2D; NKG2-D; NK cell receptor D
Size:	50 tests
Vol. per Test:	5 µl
Clone:	1D11
Immunogen:	NKL cells
Isotype:	Mouse (RBF/ DnJ) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	HLDA VIII
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 1D11 monoclonal antibody specifically binds to NKG2D, a 42 kDa type II transmembrane glycoprotein that is also known as CD314 and KLRK1. NKG2D is a member of the C-type lectin family and is expressed on human NK cells. This activating receptor binds strongly to several ligands including MICA and MICB and ULBP-1, -2, and -3 proteins that are expressed by different target cell types. Different from natural cytotoxicity receptor (NCR), NKG2D expression is not confined to NK cells. It is also expressed on virtually all TCR γ/δ+ and CD8+TCR α/β+ T cells. NKG2D functions as a triggering receptor involved in natural cytotoxicity mediated by normal NK cells against a variety of tumors or normal target cells. Importantly, NKG2D can complement the role of NCR in tumor cell lysis. Remarkably, the combined maskings of NCR and NKG2D can reportedly lead to a complete inhibition of NK-mediated lysis of all tumor or normal cells. The 1D11 antibody can reportedly block or stimulate the function of NKG2D-positive cells.



Flow cytometric analysis of CD314 expression on human peripheral blood lymphocytes. *Whole blood was stained with either PE-Cy™7 Mouse Anti-Human CD314 antibody (Cat. No. 562365; solid line histogram) or with a PE-Cy™7 Mouse IgG1, κ Isotype Control (Cat. No. 557872; dashed line histogram). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer 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.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
557872	PE-Cy™7 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
555899	Lysing Buffer	100 ml	(none)

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Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. 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.
5. 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.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. 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™ Stabilizing Fixative (Cat. No. 338036).
9. 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.
10. 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.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Bauer S, Groh V, Wu J, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science.* 1999; 285(5428):727-729. (Immunogen: Blocking, Flow cytometry, Functional assay, Immunoprecipitation, Inhibition)

Groh V, Bruhl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc Natl Acad Sci U S A.* 2003; 100(16):9452-9457. (Clone-specific: Flow cytometry, Immunohistochemistry)

Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol.* 2001; 2(3):255-260. (Clone-specific: Blocking, (Co)-stimulation, Inhibition)

Roberts AI, Lee L, Schwarz E, et al. NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. *J Immunol.* 2001; 167(10):5527-5530. (Clone-specific)

Steinle A, Li P, Morris DL, et al. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics.* 2001; 53(4):279-287. (Clone-specific: Blocking)

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