Technical Data Sheet

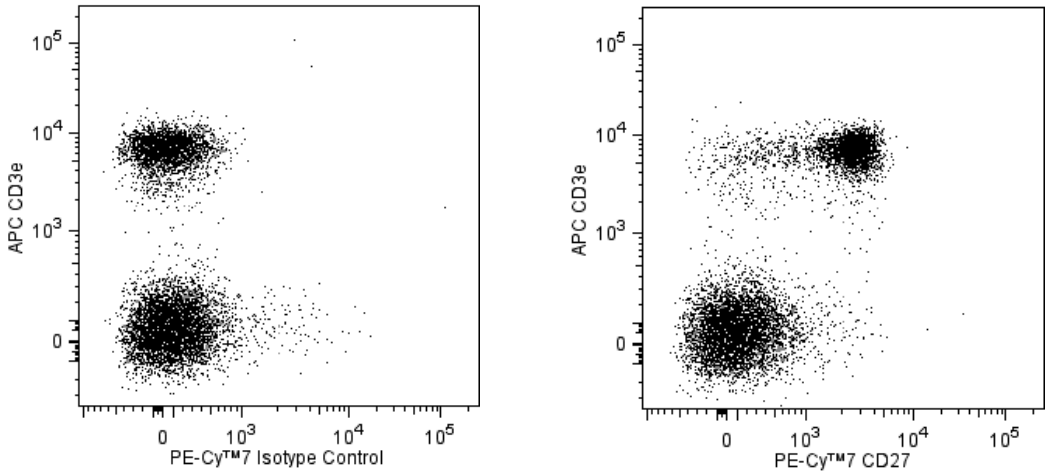
PE-Cy™7 Hamster Anti-Mouse CD27

Product Information

Material Number:	**563604**
Alternate Name:	Tnfrsf7; Tumor necrosis factor receptor superfamily member 7; Tp55; S152
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	LG.3A10
Immunogen:	Armenian hamster fibroblast line ARHO12 transfected with mouse Cd27 cDNA
Isotype:	Armenian Hamster IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Rat
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The LG.3A10 monoclonal antibody specifically binds to CD27, a lymphocyte-restricted member of the Tumor Necrosis Factor Receptor family which binds to CD70. The CD27 molecule is a 45-kDa transmembrane glycoprotein which is constitutively expressed by lymphocytes of the T lineage: virtually all thymocytes and over 90% of peripheral T cells bearing both αβ and γδ T-cell receptors. CD27 cooperates with the pre-TCR in mediating thymocyte differentiation and expansion. In addition, one to ten percent of mature peripheral B cells express CD27, and CD27's role in the differentiation of human plasma cells has been studied. Mouse NK cells, freshly isolated and IL-2-activated, also express CD27. In the bone marrow, CD27 is found on a progenitor population which provides short-term hematopoietic reconstitution. Cells of the myeloid lineage do not express CD27. Cross-linked LG.3A10 mAb has been reported to amplify the proliferative response of purified T lymphocytes to suboptimal mitogenic stimulation1 and to enhance NK-cell proliferation and IFN-γ production. In contrast, non-cross-linked LG.3A10 mAb inhibits CD3-induced pre-T cell development by interfering with the receptor-ligand interaction. This hamster mAb to a mouse leukocyte antigen has been observed to cross-react with a similar population of rat leukocytes.



Flow cytometric analysis of CD27 expression on mouse splenocytes. *Mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with APC Hamster Anti-Mouse CD3e antibody (Cat. No. 553066/561826) and either PE-Cy™7 Hamster IgG1, κ Isotype Control (Cat. No. 552811; Left Panel) or PE-Cy™7 Hamster Anti-Mouse CD27 antibody (Cat. No. 563604; Right Panel). Two-color flow cytometric dot plots showing the correlated expression patterns of CD27 (or Ig Isotype control staining) versus CD3e were derived from gated events with the forward and side light-scatter characteristics of viable leucocytes. Flow cytometric analysis 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.

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Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
552811	PE-Cy™7 Hamster IgG1, κ Isotype Control	0.1 mg	A19-3
555899	Lysing Buffer	100 ml	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
553066	APC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
561826	APC Hamster Anti-Mouse CD3e	25 µg	145-2C11

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
12. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/documents/hamster_chart_11x17.pdf.

References

Agematsu K, Hokibara S, Nagumo H, Shinozaki K, Yamada S, Komiyama A. Plasma cell generation from B-lymphocytes via CD27/CD70 interaction. *Leuk Lymphoma.* 1999; 35(3-4):219-225. (Biology)

Gravestein LA, Blom B, Nolten LA, et al. Cloning and expression of murine CD27: comparison with 4-1BB, another lymphocyte-specific member of the nerve growth factor receptor family. *Eur J Immunol.* 1993; 23(4):943-950. (Biology)

Gravestein LA, Nieland JD, Kruisbeek AM, Borst J. Novel mAbs reveal potent co-stimulatory activity of murine CD27. *Int Immunol.* 1995; 7(4):551-557. (Immunogen: (Co)-stimulation, Flow cytometry, Functional assay, Immunofluorescence, Immunoprecipitation)

Gravestein LA, van Ewijk W, Ossendorp F, Borst J. CD27 cooperates with the pre-T cell receptor in the regulation of murine T cell development. *J Exp Med.* 1996; 184(2):675-685. (Clone-specific: Flow cytometry, Immunofluorescence, Inhibition)

Takeda K, Oshima H, Hayakawa Y, et al. CD27-mediated activation of murine NK cells. *J Immunol.* 2000; 164(4):1741-1745. (Clone-specific: (Co)-stimulation, Enhancement, Flow cytometry)

Wiesmann A, Phillips RL, Mojica M, et al. Expression of CD27 on murine hematopoietic stem and progenitor cells. *Immunity.* 2000; 12(2):193-199. (Clone-specific: Flow cytometry)

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