

Technical Data Sheet

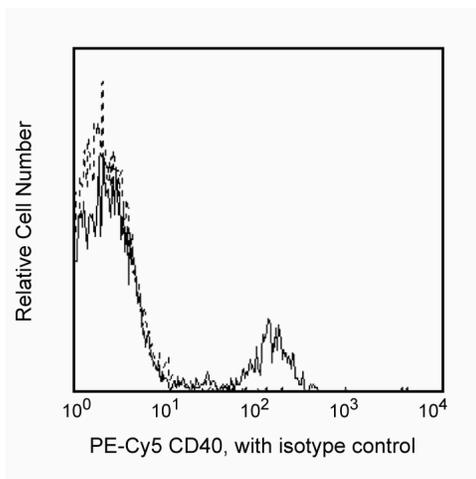
PE-Cy™5 Mouse Anti-Human CD40**Product Information**

Material Number:	555590
Alternate Name:	TNFRSF5; TNF receptor superfamily member 5; CD40L receptor; Bp50; p50
Size:	100 Tests
Vol. per Test:	20 µl
Clone:	5C3
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	V CD40.4
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

This 5C3 monoclonal antibody specifically binds to CD40, a 45-48 kDa type I integral membrane glycoprotein. CD40 is expressed on B lymphocytes, but is not expressed on terminally differentiated B cells. CD40 is also expressed by endothelial cells, basal epithelial cells and some epithelial cell carcinomas, follicular dendritic cells, macrophages, fibroblasts, keratinocytes, and CD34+ hematopoietic progenitor cells. This antibody is useful for studying the roles played by CD40 in B-cell growth, proliferation, and differentiation including immunoglobulin isotype switching. Anti-CD40 antibodies have been reported to stimulate B-cell proliferation when costimulated with anti-μ, anti-CD20 antibodies or with phorbol esters. 5C3 is capable of inducing B-cell proliferation when presented with IL-4.

Clone 5C3 reacts with the human form of the 45-48 kDa type I integral membrane glycoprotein, CD40. This clone also cross-reacts with a subset of peripheral blood lymphocytes, but not monocytes nor granulocytes, of baboon and both rhesus and cynomolgus macaque monkeys. The distribution on lymphocytes is similar to that seen with normal human donor lymphocytes, with the reactivity being restricted to CD20+ lymphocytes.



Flow cytometric analysis of CD40 expression on human peripheral blood lymphocytes. Peripheral blood lymphocytes were stained with either PE-Cy™5 Mouse IgG1 κ Isotype Control (Cat. No. 555750; dashed line histogram) or PE-Cy™5 Mouse Anti-Human CD40 (Cat. No. 555590; solid line histogram). Fluorescent histograms were derived from gated events with the side and forward light-scattering characteristics of viable lymphocytes. Flow cytometry was performed on a BD FACScan™ 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-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Application Notes**Application**

Flow cytometry

Routinely Tested

BD Biosciences

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555590 Rev. 10



Suggested Companion Products

Catalog Number	Name	Size	Clone
555750	PE-Cy TM 5 Mouse IgG1 κ Isotype Control	100 Tests	MOPC-21
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

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- μ l experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.
7. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5TM.
8. 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.
9. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc BlockTM purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc BlockTM (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.
10. Cy is a trademark of GE Healthcare.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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