# **Technical Data Sheet**

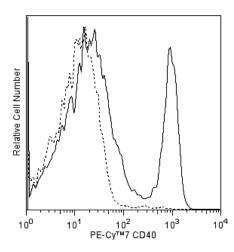
# PE-Cy<sup>™</sup>7 Mouse Anti-Human CD40

## **Product Information**

terial Number:	561215
ernate Name:	TNFRSF5; TNF receptor superfamily member 5; CD40L receptor; Bp50; p50
2:	50 tests
. per Test:	5 μl
ne:	5C3
type:	Mouse IgG1, κ
nctivity:	QC Testing: Human
rkshop:	V CD40.4
rage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.
e: . per Test: ne: type: .ctivity: rkshop:	50 tests 5 μl 5C3 Mouse IgG1, κ QC Testing: Human V CD40.4

#### 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.



Flow cytometric analysis of CD40 expression on human peripheral blood lymphocytes. Whole blood was stained with PE-Cy™7 Mouse Anti-Human CD40 antibody (Cat. No. 561215; 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 PharmLyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from gated 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**

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. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

#### **Application Notes**

Application							
Flow cytor	netry				Routinely Tested		
Suggeste	d Compani	ion Produc	ts				
Catalog Nu	nber	Name				Size	Clone
557872		PE-Cy <sup>™7</sup> Mouse IgG1 κ Isotype Control			ntrol	100 tests	MOPC-21
555899		Lysing Buffer				100 ml	(none)
554656		Stain Buffer (FBS)				500 ml	(none)
BD Bioscie	ences						
bdbiosciences.	com						
United States 877.232.8995	<b>Canada</b> 888.268.5430	Europe 32.53.720.550	<b>Japan</b> 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbean 0800.771.7157		
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### **Product Notices**

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^{6}$  cells in a 100-µl experimental sample (a test).
- 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>™</sup> Stabilizing Fixative (Cat. No. 338036).
- 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. 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.
- 6. 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.
- 7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 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. 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.
- 10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

Morio T, Hanissian SH, Bacharier LB, et al. Ku in the cytoplasm associates with CD40 in human B cells and translocates into the nucleus following incubation with IL-4 and anti-CD40 mAb. *Immunity.* 1999; 11(3):339-348. (Biology)

Nguyen LT, Duncan GS, Mirtsos C, et al. TRAF2 deficiency results in hyperactivity of certain TNFR1 signals and impairment of CD40-mediated responses. *Immunity.* 1999; 11(3):379-389. (Biology)

Randall TD, Heath AW, Santos-Argumedo L, Howard MC, Weissman IL, Lund FE. Arrest of B lymphocyte terminal differentiation by CD40 signaling: mechanism for lack of antibody-secreting cells in germinal centers. *Immunity.* 1998; 8(6):733-742. (Biology)

Schlossman SF, Boumsell L, Gilks W, et al, ed. Leukocyte Typing V: White Cell Differentiation Antigens. New York: Oxford University Press; 1995. (Clone-specific)

Stamenkovic I, Clark EA, Seed B. A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. *EMBO J.* 1989; 8(5):1403-1410. (Biology)