# **Technical Data Sheet**

# **Purified Mouse Anti-Human CD40**

#### **Product Information**

Material Number: 550391

Alternate Name: TNFRSF5; TNF receptor superfamily member 5; CD40L receptor; Bp50; p50

 Size:
 1.0 ml

 Concentration:
 125 μg/ml

 Clone:
 5C3

 $\begin{array}{lll} \textbf{Isotype:} & \textbf{Mouse IgG1, } \kappa \\ \textbf{Reactivity:} & \textbf{QC Testing: Human} \end{array}$ 

Workshop: V CD40.4

Storage Buffer: Aqueous buffered solution containing BSA, goat serum, 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- $\mu$ , anti-CD20 antibodies or with phorbol esters. 5C3 is capable of inducing B-cell proliferation when presented with IL-4.



Immunohistochemical staining of CD40+ cells. Frozen sections of normal spleen was reacted with 5C3 antibody. CD40+ lymphocytes can be identified by the intense brown labeling of their cell surface membranes. Amplification 20X.

### **Preparation and Storage**

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

### **Application Notes**

### Application

Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunohistochemistry-zinc-fixed	Tested During Development
Immunohistochemistry-paraffin	Not Recommended

## Recommended Assay Procedure:

Immunohistochemistry: The 5C3 antibody is recommended to test for immunohistochemical staining of acetone-fixed frozen sections. Tissued tested were human spleen and tonsil. The antibody stains tonsillar B lymphocytes and some lymphoid dendritic cells. The isotype control recommended for use with this antibody is purified mouse IgG1 (Cat. No. 550878). For optimal indirect immunohistochemical staining, the 5C3 antibody should be titrated (1:10 to 1:50 dilution) and visualized via a three-step staining procedure in combination with polyclonal, biotin conjugated anti-mouse Igs (multiple adsorbed) (Cat. No. 550337) as the secondary antibody and Streptravidin-HRP (Cat. No. 550946) together with the DAB detection system (Cat. No. 550880). A detailed protocol of the immunohistochemical procedure is available at our website, www.bdbiosciences.com/support/resources. The clone 5C3 is not recommended for formalin-fixed paraffin embedded sections.

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### **Suggested Companion Products**

Catalog Number	Name Name	Size	Clone	
550337	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	1.0 ml	Polyclonal	
550878	Purified Mouse IgG1 κ Isotype Control	1.0 ml	MOPC-31C	
550880	DAB Substrate Kit	500 tests	(none)	
550946	Streptavidin HRP	50 ml	(none)	
551011	Anti-Mouse Ig HRP Detection Kit	200 tests	(none)	
559148	Antibody Diluent for IHC	125 ml	(none)	

#### **Product Notices**

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 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.
- 3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 4. This antibody has been developed for the immunohistochemistry application. However, a routine immunohistochemistry test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
- 5. An isotype control should be used at the same concentration as the antibody of interest.
- 6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

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Galy AH, Spits H. CD40 is functionally expressed on human thymic epithelial cells. J Immunol. 1992; 149(3):775-782. (Biology)

Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell. 1991; 66(2):233-243. (Biology)

Schlossman S, Boumell L, et al, ed. *Leucocyte Typing V*. New York: Oxford University Press; 1995. (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)

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