

## Technical Data Sheet

## Alexa Fluor® 700 Mouse Anti-Human CD40

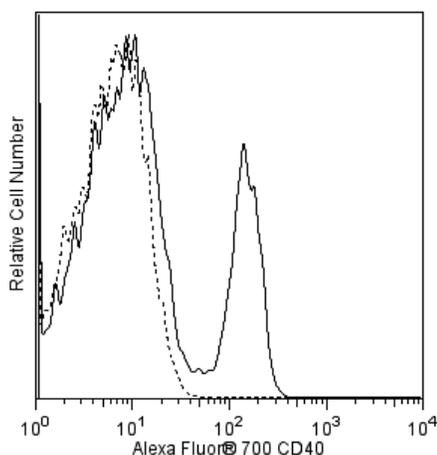
## Product Information

<b>Material Number:</b>	<b>561208</b>
<b>Alternate Name:</b>	TNFRSF5; TNF receptor superfamily member 5; CD40L receptor; Bp50; p50
<b>Size:</b>	50 Tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	5C3
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
<b>Workshop:</b>	V CD40.4
<b>Storage Buffer:</b>	Aqueous buffered solution containing protein stabilizer 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 lymphocytes.** Whole blood was stained with Alexa Fluor® 700 Mouse Anti-Human CD40 antibody (Cat. No. 561208; solid line histogram) or with a Alexa Fluor® 700 Mouse IgG1, κ Isotype Control (Cat. No. 557882; dashed line histogram). The erythrocytes were lysed with BD PharmLyse™ 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

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

The antibody was conjugated to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

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

## Application Notes

## Application

Flow cytometry

Routinely Tested

## BD Biosciences

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## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
557882	Alexa Fluor® 700 Mouse IgG1, $\kappa$ Isotype Control	0.1 mg	MOPC-21
555899	Lysing Buffer	100 mL	(none)
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  $1 \times 10^6$  cells in a 100- $\mu$ l experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
8. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
9. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

## References

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- 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)
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- 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)