Technical Data Sheet

PE-Cy™7 Mouse Anti-Human CD40

Product Information

Material Number: 561215
Alternate Name: TNFRSF5; TNF receptor superfamily member 5; CD40L receptor; Bp50; p50
Size: 50 Tests
Vol. per Test: 5 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. 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

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.

Application Notes

Application

| Flow cytometry | Routinely Tested |

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### Suggested Companion Products

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<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>557872</td>
<td>PE-Cy™7 Mouse IgG1 κ Isotype Control</td>
<td>100 Tests</td>
<td>MOPC-21</td>
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<tr>
<td>555899</td>
<td>Lysing Buffer</td>
<td>100 mL</td>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<td>554657</td>
<td>Stain Buffer (BSA)</td>
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</tbody>
</table>

### 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. 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. 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).
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. 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.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Cy is a trademark of GE Healthcare.
10. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

### References

- 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)