

DESCRIPTION

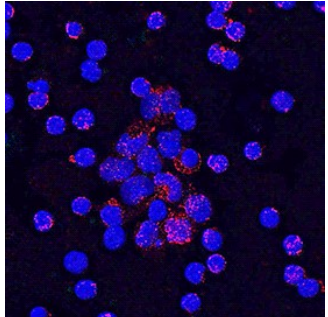
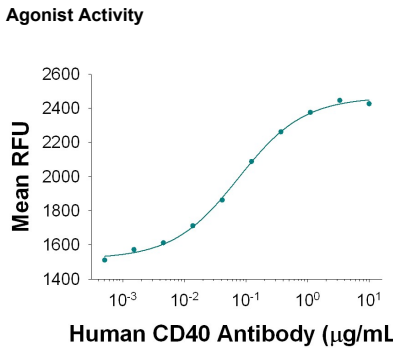
Species Reactivity	Human
Specificity	Detects human CD40/TNFRSF5 in direct ELISAs and Western blots. In direct ELISAs, does not cross-react with recombinant human (rh) 4-1BB, rhCD27, rhCD30, recombinant mouse CD40, rhDR3, rhDR6, rhEDAR, rhFas, rhGITR, rhHVEM, rhLTRβ, rhNGF R, rhOPG, rhRANK, rhTAJ, rhTNF RI, or rhTNF RII.
Source	Monoclonal Mouse IgG _{2B} Clone # 82111
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human CD40/TNFRSF5 Glu21-Arg193 Accession # P25942
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Flow Cytometry	2.5 µg/10 ⁶ cells	Human whole blood CD19 ⁺ B cells
Immunocytochemistry	8-25 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Agonist Activity	Measured in a cell proliferation assay using B cell enriched human peripheral blood lymphocytes in the presence of IL-4. Banchereau, J. <i>et al.</i> (1991) <i>Science</i> 251 :70. The ED ₅₀ for this effect is typically 0.035-0.175 µg/mL.	

DATA

<p>Immunocytochemistry</p>  <p>CD40/TNFRSF5 in Human PBMCs. CD40/TNFRSF5 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Mouse Anti-Human CD40/TNFRSF5 Monoclonal Antibody (Catalog # MAB6321) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces and cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>	<p>Agonist Activity</p>  <p>Human CD40/TNFRSF5 Antibody Stimulates Cell Proliferation in Human B Cells. Mouse Anti-Human CD40/TNFRSF5 Monoclonal Antibody (Catalog # MAB6321) stimulates human B cell proliferation in the presence of Recombinant Human IL-4 (Catalog # 204-IL) in a dose-dependent manner, as measured by Resazurin (Catalog # AR002). The ED₅₀ for this effect is typically 0.035-0.175 µg/mL.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

CD40 is a type I transmembrane glycoprotein belonging to the TNF receptor superfamily. The mature hCD40 consists of a 172 amino acid (aa) extracellular domain, a 22 aa transmembrane region and a 62 aa cytoplasmic domain (1). Human and mouse CD40 share 62% aa identity. CD40 is expressed in B cells, follicular dendritic cells, dendritic cells, activated monocytes, macrophages, endothelial cells, vascular smooth muscle cells, and several tumor cell lines (2). The extracellular domain has the cysteine-rich repeat regions, which are characteristic for many of the receptors of the TNF superfamily. Interaction of CD40 with its ligand, CD40L, leads to aggregation of CD40 molecules, which in turn interact with cytoplasmic components to initiate signaling pathways. Early studies on the CD40-CD40L system revealed its role in humoral immunity. Interaction between CD40L on T cells and CD40 on B cells stimulated B cell proliferation and provided the signal for immunoglobulin isotype switching (3). Mutations in the CD40L gene, which resulted in a CD40L molecule unable to interact with CD40, are responsible for the hyper-IgM syndrome (4). Cross-linking of CD40 with antibodies or by CD40 binding to CD40L produces cell type-specific responses which include costimulation and induction of proliferation, induction of cytokine production, rescue from apoptosis, and upregulation of adhesion molecules (5). Some of the early events of intracellular signaling by the CD40-CD40L system include the association of the CD40 with TRAFs and the activation of various kinases (6-8).

References:

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2. Schonbeck, U. *et al.* (1997) *J. Biol. Chem.* **272**:19569.
3. Armitage, R.J. *et al.* (1993) *J. Immunol.* **150**:3671.
4. Callard, R.E. *et al.* (1993) *Immunol. Today* **14**:559.
5. Stout, R.D. and J. Suttles (1996) *Immunol. Today* **17**:487.
6. Pullen, S.S. *et al.* (1999) *Biochemistry* **38**:10168.
7. Faris, M. *et al.* (1994) *J. Exp. Med.* **179**:1923.
8. Hanissian, S.H. and R.S. Geha (1997) *Immunity* **6**:379.