

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD40 in direct ELISAs and Western blots. In direct ELISAs, less than 5% cross-reactivity with recombinant mouse CD40 and recombinant rat CD40 is observed, and less 1% cross-reactivity with recombinant human (rh) TNF RI, rhTNF RII, rhCD40 Ligand, rh4-1BB, rhCD27, rhCD30, rhDR3, rhDR6, rhFas, rhGITR, rhHVEM, rhNGF R, rhRANK and rhREL1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human CD40 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 as a 0.2 µm filtered solution in PBS.

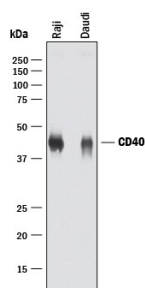
APPLICATIONS

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
Western Blot	2 µg/mL	See Below
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 3-12 ng/mL.	

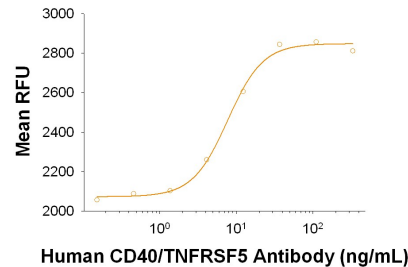
DATA

Western Blot



Detection of Human CD40/TNFRSF5 by Western Blot. Western blot shows lysates of Raji human Burkitt's lymphoma cell line and Daudi human Burkitt's lymphoma cell line. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human CD40/TNFRSF5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF632) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CD40/TNFRSF5 at approximately 40-45 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

Agonist Activity



Human CD40/TNFRSF5 Antibody Stimulates Cell Proliferation in Human B Cells. Goat Anti-Human CD40/TNFRSF5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF632) 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 3-12 ng/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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:

1. Torres, R.M. and E.A. Clark (1992) *J. Immunol.* **148**:620.
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.