

## DESCRIPTION

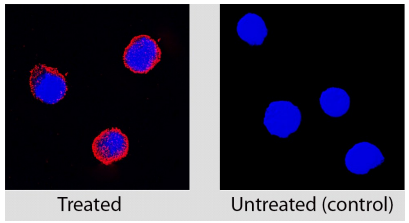
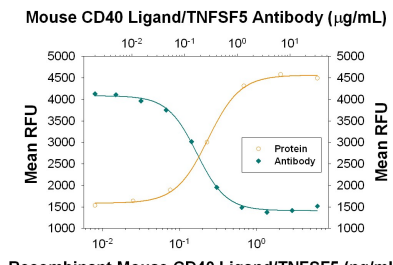
<b>Species Reactivity</b>	Mouse
<b>Specificity</b>	Detects mouse CD40 Ligand/TNFSF5 in Western blots.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant mouse CD40 Ligand/TNFSF5 Glu61-Leu260 Accession # P27548
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	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
<b>Western Blot</b>	0.1 µg/mL	Recombinant Mouse CD40 Ligand/TNFSF5 (Catalog # 1163-CL)
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Neutralization</b>	Measured by its ability to neutralize CD40 Ligand/TNFSF5-induced proliferation in mouse splenic B cells. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.08-0.4 µg/mL in the presence of 0.5 ng/mL Recombinant Mouse CD40 Ligand/TNFSF5.	

## DATA

<p><b>Immunocytochemistry</b></p>  <p><b>CD40 Ligand/TNFSF5 in Mouse Splenocytes.</b> CD40 Ligand/TNFSF5 was detected in immersion fixed mouse splenocytes either stimulated with PHA (left panel) or untreated (right panel) using Goat Anti-Mouse CD40 Ligand/TNFSF5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1163) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for <a href="#">Fluorescent ICC Staining of Non-adherent Cells</a>.</p>	<p><b>Neutralization</b></p>  <p><b>Proliferation Induced by CD40 Ligand/TNFSF5 and Neutralization by Mouse CD40 Ligand/TNFSF5 Antibody.</b> Recombinant Mouse CD40 Ligand/TNFSF5 (Catalog # 8230-CL) induces proliferation in mouse splenic B cells in a dose-dependent manner (orange line), as measured by Resazurin (Catalog # AR002). Proliferation elicited by Recombinant Mouse CD40 Ligand/TNFSF5 (0.5 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Mouse CD40 Ligand/TNFSF5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1163). The ND<sub>50</sub> is typically 0.08-0.4 µg/mL.</p>
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## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

CD40 ligand (CD40L), also known as CD154, TNFSF5, TRAP or gp39, is a 260 amino acid (aa) type II transmembrane glycoprotein belonging to the TNF family. Murine CD40L consists of a 22 aa cytoplasmic domain, a 24 aa transmembrane domain, and 214 aa extracellular domain bearing a single glycosylation site (1, 2). CD40L is expressed predominantly on activated CD4<sup>+</sup> T lymphocytes, and also found in other types of cells, including NK cells, mast cells, basophils and eosinophils. Murine CD40L shares 78% amino acid sequence identity with human CD40L. Native bioactive soluble CD40L exists. Soluble human trimeric CD40L secreted by stimulated T cells has been shown to be generated by proteolysis in the microsomes (3). Both membrane bound and soluble CD40L induce similar effects on B cells (3, 4). The receptor of CD40L is CD40, a type I transmembrane glycoprotein belonging to the TNF receptor family. CD40 is expressed on B lymphocytes, monocytes, dendritic cells, and thymic epithelium. Although all monomeric, dimeric and trimeric forms of soluble CD40L can bind to CD40, the soluble trimeric form of CD40L has the most potent biological activity through oligomerization of cell surface CD40, a common feature of TNF receptor family members (2). The genetic defect in the hyper-IgM syndrome is due to point mutations or deletions of the gene encoding the CD40L, which prevent CD40L from interacting with CD40 (5-7). CD40L mediates a range of activities on B cells including induction of activation-associated surface antigen, entry into the cell cycle, isotype switching, Ig secretion, and memory generation (8, 9). CD40-CD40L interaction also plays important roles in monocyte activation and dendritic cell maturation (10).

**References:**

1. Armitage, R.J. *et al.* (1992) *Nature* **357**:80.
2. Hollenbaugh, D. *et al.* (1992) *EMBO J.* **11**:4313.
3. Fabienne, P. *et al.* (1996) *J. Biol. Chem.* **271**:5965.
4. Fabienne, P. *et al.* (1996) *Eur. J. Immunol* **26**:725.
5. Arrufo, A. *et al.* (1993) *Cell* **72**:291.
6. Hill, A. and N. Chapel *et al.* (1993) *Nature* **361**:494.
7. Korthauer, U. *et al.* (1993) *Nature* **361**:539.
8. Spriggs, M.K. *et al.* (1992) *J. Exp. Med.* **176**:1543.
9. Fanslow, W.C. *et al.* (1994) *Seminars in Immunology* **6**:267.
10. Kooten, C.V. and J. Banchereau (2000) *J. Leukoc. Biol.* **67**:2.