Mouse CD40 Ligand/TNFSF5 Antibody

Monoclonal Rat IgG_{2A} Clone # 208109

Catalog Number: MAB1163

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse CD40 Ligand in direct ELISAs. In direct ELISAs, no cross-reactivity with recombinant mouse (rm) CD27 Ligand, rmCD30 Ligand, or recombinant human CD40 Ligand is observed.
Source	Monoclonal Rat IgG_{2A} Clone # 208109
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse CD40 Ligand/TNFSF5 Glu61-Leu260 Accession # P27548
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.

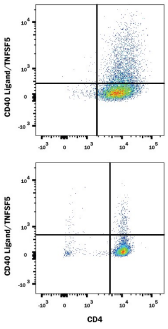
APPLICATIONS

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	0.25 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize CD40 Ligand/TNFSF5-induced proliferation in mouse splenic B cells. The Neutralization Dose (ND_{50}) is typically 0.0075-0.045 µg/mL in the presence of 0.5 ng/mL Recombinant Mouse CD40 Ligand/TNFSF5.	

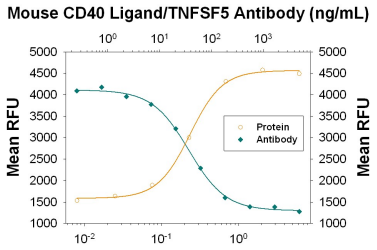
DATA

Flow Cytometry



Detection of CD40 Ligand/TNFSF5 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes either (A) stimulated to induce Th1 cells or (B) unstimulated were stained with Rat Anti-Mouse CD40 Ligand/TNFSF5 Monoclonal Antibody (Catalog # MAB1163) followed by Allophycocyanin-conjugated Anti-Rat IgG Secondary Antibody (Catalog # F0113) and Rat Anti-Mouse CD4 PE-conjugated Monoclonal Antibody (Catalog # FAB554P). Quadrant markers were set based on control antibody staining (Catalog # MAB006). View our protocol for Staining Membrane-associated Proteins.

Neutralization



Proliferation Induced by CD40 Ligand/TNFSF5 and Neutralization by Mouse CD40 Ligand/TNFSF5 Antibody. 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 Rat Anti-Mouse CD40 Ligand/TNFSF5 Monoclonal Antibody (Catalog # MAB1163). The ND_{50} is typically 0.0075-0.045 µg/mL.

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.** ● 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.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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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. Mouse 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 $CD4^+$ 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:

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