

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse OX40 Ligand/TNFSF4 in direct ELISAs and Western blots. In Western blots, approximately 5% cross-reactivity with recombinant human (rh) OX40 Ligand, rhLIGHT, recombinant mouse (rm) Fas Ligand and less than 1% cross-reactivity with rmTNF- α , rhGTR Ligand, rhTRANCE, and rhVEGI is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse OX40 Ligand/TNFSF4 Gln49-Leu198 Accession # P43488
Endotoxin Level	<0.10 EU per 1 μ g of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 μ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μ m filtered solution in PBS.

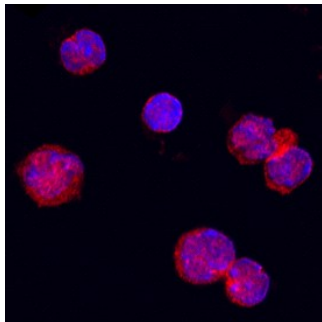
APPLICATIONS

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	0.1 μ g/mL	Recombinant Mouse OX40 Ligand/TNFSF4 (Catalog # 1236-OX)
Immunocytochemistry	5-15 μ g/mL	See Below
Neutralization	Measured by its ability to neutralize OX40 Ligand/TNFSF4-induced IL-2 secretion in mouse T cells. The Neutralization Dose (ND ₅₀) is typically 0.2-0.6 μ g/mL in the presence of 100 ng/mL Recombinant Mouse OX40 Ligand/TNFSF4, 10 μ g/mL a cross-linking antibody, Mouse polyHistidine Monoclonal Antibody, and sub-optimal amounts of Mouse CD3 ϵ Monoclonal Antibody.	

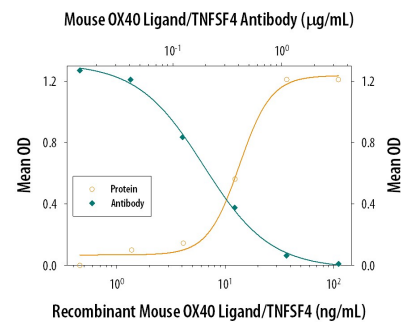
DATA

Immunocytochemistry



OX40 Ligand/TNFSF4 in Mouse Splenocytes. OX40 Ligand/TNFSF4 was detected in immersion fixed mouse splenocytes using Goat Anti-Mouse OX40 Ligand/TNFSF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1236) at 15 μ g/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 the plasma membrane and cytoplasm. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

Neutralization



IL-2 Secretion Induced by OX40 Ligand/TNFSF4 and Neutralization by Mouse OX40 Ligand/TNFSF4 Antibody. In the presence of a cross-linking antibody, Mouse polyHistidine Monoclonal Antibody (10 μ g/mL, Catalog # MAB050) and sub-optimal amounts of Mouse CD3 ϵ Monoclonal Antibody (Catalog # MAB484), Recombinant Mouse OX40 Ligand/TNFSF4 (Catalog # 1236-OX) stimulates IL-2 secretion in mouse T cells in a dose-dependent manner (orange line), as measured by the Mouse IL-2 Quantikine ELISA Kit (Catalog # M2000). Under these conditions, IL-2 secretion elicited by Recombinant Mouse OX40 Ligand/TNFSF4 (100 ng/mL) is neutralized (green line) by increasing concentrations of Mouse OX40 Ligand/TNFSF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1236). The ND₅₀ is typically 0.2-0.6 μ g/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

OX40 Ligand (OX40L), also known as gp34, is a type II transmembrane glycoprotein belonging to the TNF superfamily. Murine OX40L cDNA encodes a 198 amino acid (aa) residue protein comprised of a 28 aa N-terminal cytoplasmic domain, a 20 aa transmembrane segment, and a 150 aa C-terminal extracellular domain (1). Human and murine OX40L share 46% sequence identity at the amino acid level (1). The OX40L is expressed on activated antigen presenting cells such as B cells, macrophages, dendritic cells, and on endothelial cells at the site of inflammation. The receptor for OX40L is OX40 (CD134) that is expressed predominantly on activated CD4⁺ T cells. Expression of OX40 is transient following engagement of T cell receptors (2). Ligation of OX40L by OX40 stimulates proliferation and differentiation of activated B cells, and increases immunoglobulin secretion (3, 4). The expression of OX40L on B cells is up-regulated by CD40 ligation (3). Engagement of the OX40-OX40L system has co-stimulatory effects on T cells by stimulating the production of cytokines by T helper cells and increasing the survival of memory T cells (2, 5). Blocking of the OX40-OX40L interaction *in vitro* inhibits co-stimulation resulting in decreased T cell proliferation and adhesion of T cells to endothelial cells. Inhibition of the OX40-OX40L interaction in disease models has beneficial effects in acute graft-versus-host disease, inflammatory bowel disease, and decreases the development of collagen-induced arthritis and experimental leishmaniasis (6).

References:

1. Baum, P.R. *et al.* (1994) EMBO J. **13**:3992.
2. Gramaglia, I. *et al.* (1999) J. Immunol. **161**:6510.
3. Stuber, E. *et al.* (1995) Immunity **2**:507.
4. Malstrom, V. *et al.* (2001) J. Immunol. **166**:6972.
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6. Weinberg, A.D. (2002) Trends Immunol. **23**:102.