Species Reactivity: Human

Specificity: Detects human OX40 Ligand in direct ELISAs. In direct ELISAs, no cross-reactivity with recombinant human (rh) APRIL, rhBAFF, rhCD27 Ligand, recombinant mouse (rm) CD27 Ligand, rhCD30 Ligand, rmCD30 Ligand, rhCD40 Ligand, rmCD40 Ligand, rmGITR Ligand, recombinant cotton rat TNF-α, rhTNF-α, rmTNF-α, recombinant porcine TNF-α, recombinant rat TNF-α, rhTRAIL, rhTRANCE, rmTRANCE, or rhVEGF is observed.

Source: Monoclonal Mouse IgG1 Clone # 159403

Purification: Protein A or G purified from hybridoma culture supernatant

Immunogen: Mouse myeloma cell line NS0-derived recombinant human OX40 Ligand

Gln51-Leu183

Accession # P23510

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Flow Cytometry</th>
<th>Immunocytochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 μg/10⁶ cells</td>
<td>See Below</td>
<td>See Below</td>
</tr>
</tbody>
</table>

Neutralization: Measured by its ability to neutralize OX40 Ligand/TNFSF4-induced IL-8 secretion in the HT1080 human fibrosarcoma cell line transfected with human OX40. Muller, N. et al. (2008) FEBS Journal 275:2296. The Neutralization Dose (ND₅₀) is typically 0.1-0.6 μg/mL in the presence of 10 ng/mL Recombinant Human OX40 Ligand/TNFSF4.

DATA

Flow Cytometry: Detection of OX40 Ligand/TNFSF4 in Human Mature Dendritic Cells by Flow Cytometry. Human mature dendritic cells differentiated from human peripheral blood mononuclear cell derived CD14⁺ cells treated with 20 ng/mL Recombinant Human IL-4 (Catalog # 204-L) and 50 ng/mL Recombinant Human GM-CSF (Catalog # 215-GM) for 7 days and 1 μg/mL LPS, 20 ng/mL Recombinant Human TNF-α (Catalog # 200-TA), and 20 ng/mL Recombinant Human IL-1β/IL-1F2 (Catalog # 201-LB) for last 24 hours were stained with Mouse Anti-Human OX40 Ligand/TNFSF4 Monoclonal Antibody (Catalog # MAB10541, filled histogram) or isotype control (Catalog # MAB002, open histogram), followed by Goat F(ab')2 Anti-Mouse IgG (H+L) Allophycocyanin (Catalog # F0101B).

Immunocytochemistry: OX40 Ligand/TNFSF4 in Human PBMCs. OX40 Ligand/TNFSF4 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) stimulated with LPS using Mouse Anti-Human OX40 Ligand/TNFSF4 Monoclonal Antibody (Catalog # MAB10541) at 25 μg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (orange; Catalog # NL007) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.
**Neutralization**

IL-8 Secretion Induced by Recombinant Human OX40 Ligand/TNFSF4 antibodies (Catalog # 1054-OX) induces IL-8 secretion in the HT1080 human fibrosarcoma cell line transfected with human OX40 in a dose-dependent manner (orange line) as measured by Human IL-8 Duoset (Catalog # DY208). IL-8 secretion elicited by Recombinant Human OX40 Ligand/TNFSF4 (10 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human OX40 Ligand/TNFSF4 Monoclonal Antibody (Catalog # MAB10541). The ND$_{50}$ is typically 0.1-0.6 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.

**BACKGROUND**

OX40 Ligand (OX40L), also known as gp34, is a type II transmembrane glycoprotein designated TNFSF4 within the TNF superfamily. Human OX40L cDNA encodes a 183 amino acids (aa) polypeptide with an amino-terminal cytoplasmic domain (aa 1-23) and a carboxy-terminal extracellular domain (aa 51-183). It shares 46% aa sequence identity with the mouse counterpart. OX40L is expressed on the surface of activated B cells, T cells, dendritic cells and endothelial cells. Like other TNF superfamily members, membrane-bound OX40 Ligand exists as a homotrimer. OX40L binds to OX40 (CD134), a member of the TNF receptor superfamily that is expressed predominantly on activated CD4$^+$ T cells. OX40 Ligand is one of the group of co-stimulatory molecules in the immune system that includes B7, CD40 Ligand, CD30 Ligand, CD27 Ligand and 4-1BB Ligand. OX40 appears as a late activation-induced T cell surface antigen, and its major function of OX40-OX40L interaction may be to transmit a late co-stimulatory signal to promote the survival and proliferation of activated CD4$^+$ T cells and prolong the immune response. Engagement of OX40 on activated T cells in situ in tumors has been shown to augment immune responses and subsequent tumor regression.

**References:**