

## DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	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, mEDA, rhFas Ligand, rmFas Ligand, rhGITR Ligand, recombinant cotton rat TNF- $\alpha$ , rhTNF- $\alpha$ , rmTNF- $\alpha$ , recombinant porcine TNF- $\alpha$ , recombinant rat TNF- $\alpha$ , rhTRAIL, rhTRANCE, rmTRANCE, or rhVEGF is observed.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 159403
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human OX40 Ligand Gln51-Leu183 Accession # P23510
<b>Endotoxin Level</b>	<0.10 EU per 1 $\mu$ g of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m 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 $\mu$ m filtered solution in PBS.

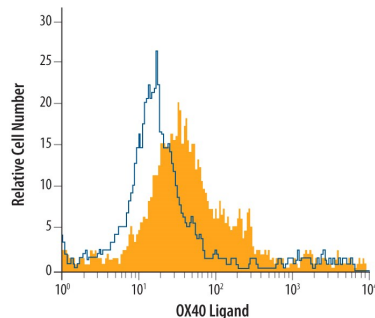
## APPLICATIONS

**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
<b>Flow Cytometry</b>	2.5 $\mu$ g/10 <sup>6</sup> cells	See Below
<b>Immunocytochemistry</b>	8-25 $\mu$ g/mL	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
<b>Neutralization</b>	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 (ND <sub>50</sub> ) is typically 0.1-0.6 $\mu$ g/mL in the presence of 10 ng/mL Recombinant Human OX40 Ligand/TNFSF4.	

## DATA

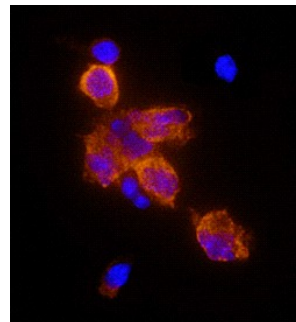
### Flow Cytometry



#### Detection of OX40 Ligand/TNFSF4 in Human Mature Dendritic Cells by Flow Cytometry.

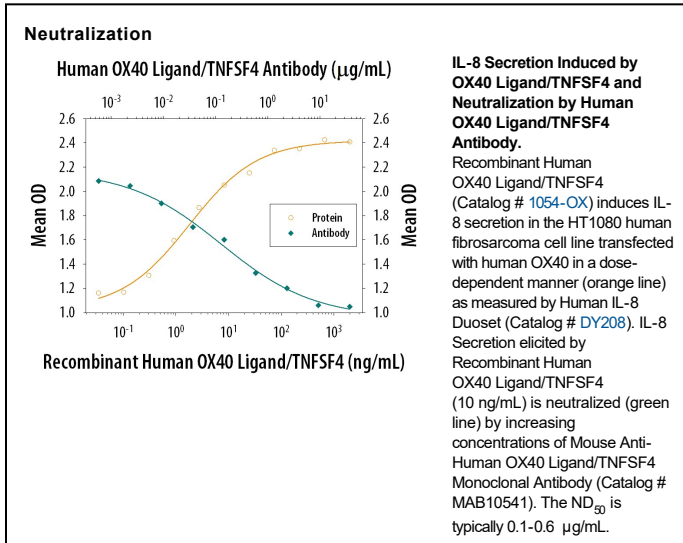
Human mature dendritic cells differentiated from human peripheral blood mononuclear cell derived CD14<sup>+</sup> cells treated with 20 ng/mL Recombinant Human IL-4 (Catalog # 204-IL) and 50 ng/mL Recombinant Human GM-CSF (Catalog # 215-GM) for 7 days and 1  $\mu$ g/mL LPS, 20 ng/mL Recombinant Human TNF- $\alpha$  (Catalog # 210-TA), and 20 ng/mL Recombinant Human IL-1 $\beta$ /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  $\mu$ g/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](#).



## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 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>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <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

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<sup>+</sup> 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<sup>+</sup> 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:

1. Godfrey, W.R. *et al.* (1994) J. Exp. Med. **180**:757.
2. Baum, P.R. *et al.* (1994) EMBO J. **13**:3992.
3. Al-Shamkhani, A. *et al.* (1997) J. Biol. Chem. **272**:5275.
4. Kjaergaard, J. *et al.* (2000) Cancer Res. **60**:5514.