

## DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human OX40/TNFRSF4 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinant mouse OX40 is observed.
<b>Source</b>	Polyclonal Sheep IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human OX40 Leu29-Ala216 Accession # P43489
<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 either lyophilized or as a 0.2 µm filtered solution in PBS.

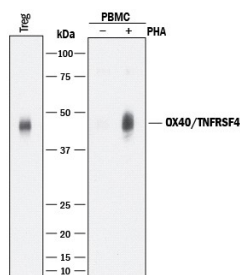
## APPLICATIONS

**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
<b>Western Blot</b>	0.5 µg/mL	See Below
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>Immunohistochemistry</b>	3-15 µg/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.	

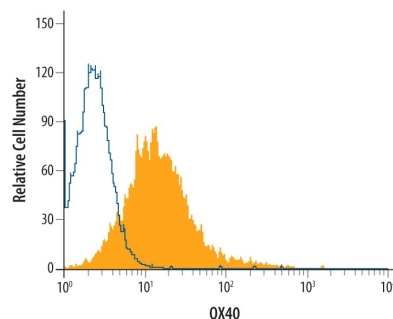
## DATA

### Western Blot



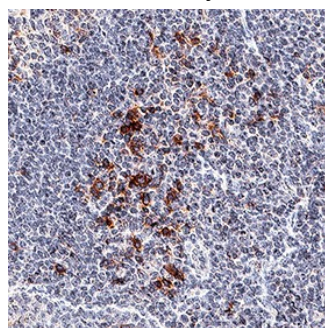
**Detection of Human OX40/TNFRSF4 by Western Blot.** Western blot shows lysates of human T regulatory cells (Tregs) and human peripheral blood mononuclear cells (PBMCs) untreated (-) or treated (+) with 1 µg/mL PHA for 5 days. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human OX40/TNFRSF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3388) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for OX40/TNFRSF4 at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

### Flow Cytometry



**Detection of OX40/TNFRSF4 in Human T Cells by Flow Cytometry.** Human T cells were treated for 3-5 days with 1 µg/mL PHA then stained with Sheep Anti-Human OX40/TNFRSF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3388, filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL010).

### Immunohistochemistry



**OX40/TNFRSF4 in Human Tonsil.** OX40/TNFRSF4 was detected in immersion fixed paraffin-embedded sections of human tonsil using Sheep Anti-Human OX40/TNFRSF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3388) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Sheep IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC006). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

## 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>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <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 (CD134; TNFRSF4) is a T cell costimulatory molecule of the TNF receptor superfamily that coordinates with other membrane-bound costimulators such as CD28, CD40, CD30, CD27 and 4-1BB (1-3). OX40 is expressed on naïve CD4<sup>+</sup> T cells only after engagement of the TCR by antigen presenting cells (APC; dendritic and B cells), and costimulation by CD40/CD40 ligand and CD28/B7. It is maximal at 2-5 days post activation, or 4 hours post reactivation of memory T cells (3-6). Human OX40 is a 48 kDa type I transmembrane glycoprotein with a 28 amino acid (aa) signal sequence, a 185 aa extracellular domain (ECD) that has four TNFR-Cys repeats and an O-glycosylated hinge region, a 20 aa transmembrane segment, and a 41 aa cytoplasmic domain (3). The ECD of human OX40 shows 71%, 68%, 67%, 64% and 64% aa identity with feline, canine, rabbit, mouse and rat OX40 ECD, respectively. Engagement of OX40 on activated CD4<sup>+</sup> T cells by OX40 ligand on activated dendritic cells promotes T cell survival and proliferation, prolongs the immune response, and enhances the number of cells making the transition from effector to memory T cells (1-6). OX40 signal transduction includes binding TNF receptor-associated factors (TRAFs), and activating NFκB and PI3 kinase to enhance expression of cytokines, antiapoptotic Bcl-2 family members, survivin and the chemokine receptor CXCR5 (5-8). CXCR5 promotes T cell migration to germinal centers to deliver B cell help (5). Studies using knockout or transgenic mice, and agonistic or blocking antibodies, show that OX40/OX40L interaction is critical for establishing or reactivating memory T cells and breaking immune tolerance (9). Blockade of OX40 engagement is efficacious in animal models of allergic airway inflammation, graft-versus-host disease and autoimmune disease (10-14).

**References:**

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