Human OX40/TNFRSF4 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF3388

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human OX40/TNFRSF4 in direct ELISAs and Western blots. In direct ELISAs, less than 5% cross-reactivity with recombinant mouse OX40 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human OX40 Leu29-Ala216 Accession # P43489
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.

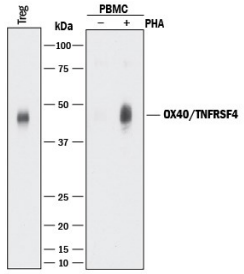
APPLICATIONS

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
Western Blot	0.5 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	3-15 µg/mL	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 OX40L/TNFSF4-induced IL-8 secretion in the HT1080 human fibrosarcoma cell line transfected with human OX40/TNFRSF4. The Neutralization Dose (ND_{50}) is typically 0.025-0.16 µg/mL in the presence of 10 ng/mL Recombinant Human OX40L/TNFSF4.	

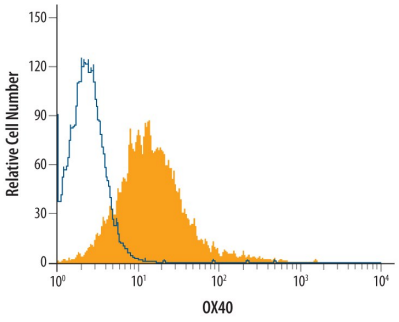
DATA

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).

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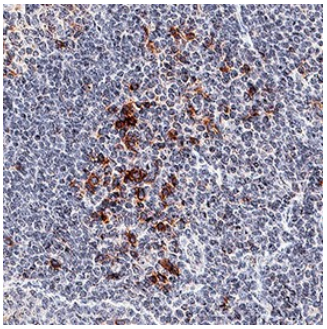
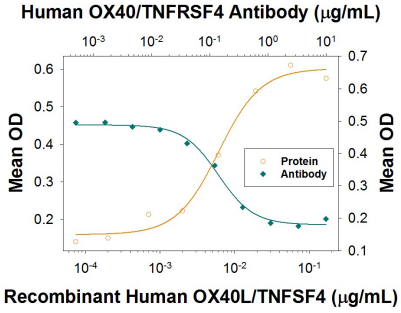
Immunohistochemistry



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.

Neutralization



CXCL8/IL-8 Secretion Induced by OX40L/TNFSF4 and Neutralization by Human OX40/TNFRSF4 Antibody. Recombinant Human OX40L/TNFSF4 (Catalog # 1054-OX) stimulates CXCL8/IL-8 secretion in the HT1080 human fibrosarcoma cell line transfected with human OX40/TNFRSF4 in a dose-dependent manner (orange line), as measured by the Human CXCL8/IL-8 Quantikine ELISA Kit (Catalog # D8000C). CXCL8/Il-8 Secretion elicited by Recombinant Human OX40L/TNFSF4 (10 ng/mL) is neutralized (green line) by increasing concentrations of Sheep Anti-Human OX40/TNFRSF4 Polyclonal Antibody (Catalog # AF3388). The ND_{50} is typically 0.025-0.16 µg/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.** • 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 (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 $CD4^+$ 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 $CD4^+$ 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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