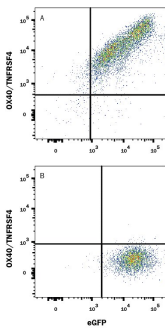
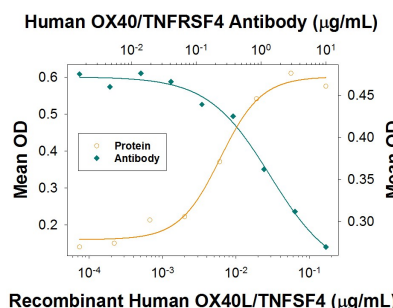


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human OX40/TNFRSF4 in direct ELISAs
Source	Monoclonal Mouse IgG _{2B} Clone # 977974
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human OX40/TNFRSF4 Leu29-Ala216 Accession # P43489
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. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.	
	Recommended Concentration Sample
Flow Cytometry	0.25 µg/10 ⁶ cells 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 0.6-3.0 µg/mL in the presence of 10 ng/mL Recombinant Human OX40L/TNFSF4.

DATA	
<p>Flow Cytometry</p>  <p>Detection of OX40/TNFRSF4 in HEK293 Human Cell Line Transfected with Human OX40/TNFRSF4 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with (A) human OX40/TNFRSF4 or (B) irrelevant transfectants and eGFP was stained with Mouse Anti-Human OX40/TNFRSF4 Monoclonal Antibody (Catalog # MAB10543) followed by APC-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). Quadrant markers were set based on control antibody staining (Catalog # MAB0041). View our protocol for Staining Membrane-associated Proteins.</p>	<p>Neutralization</p>  <p>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 Mouse Anti-Human OX40/TNFRSF4 Monoclonal Antibody (Catalog # MAB10543). The ND₅₀ is typically 0.6-3.0 µg/mL.</p>

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. <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 (CD134; TNFRSF4) is a T cell co-stimulatory molecule of the TNF receptor superfamily that coordinates with other membrane-bound co-stimulators 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 co-stimulation 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:

1. Salek-Ardakani, S. and M. Croft (2006) *Vaccine* **24**:872.
2. Hori, T. (2006) *Int. J. Hematol.* **83**:17.
3. Latza, U. *et al.* (1994) *Eur. J. Immunol.* **24**:677.
4. Murata, K. *et al.* (2000) *J. Exp. Med.* **191**:365.
5. Fillatreau, S and D. Gray (2003) *J. Exp. Med.* **197**:195.
6. Gramaglia, I. *et al.* (1998) *J. Immunol.* **161**:6510.
7. Rogers, P.R. *et al.* (2001) *Immunity* **15**:445.
8. Song, J. *et al.* (2005) *Immunity* **22**:621.
9. Bansal-Pakala, P. *et al.* (2001) *Nat. Med.* **7**:907.
10. Salek-Ardakani, S. *et al.* (2003) *J. Exp. Med.* **198**:315.
11. Jember, A. G. *et al.* (2001) *J. Exp. Med.* **193**:387.
12. Demirci, G. *et al.* (2004) *J. Immunol.* **172**:1691.
13. Blazar, B.R. *et al.* (2003) *Blood* **101**:3741.
14. Higgins, L.M. *et al.* (1999) *J. Immunol.* **162**:486.