



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH10-17

Description: The protein encoded by this gene is a member of the TNF-receptor superfamily. This receptor has been shown to activate NF-kappaB through its interaction with adaptor proteins TRAF2 and TRAF5. Knockout studies in mice suggested that this receptor promotes the expression of apoptosis inhibitors BCL2 and BCL2L1/BCL2-XL, and thus suppresses apoptosis. The knockout studies also suggested the roles of this receptor in CD4+ T cell response, as well as in T cell-dependent B cell proliferation and differentiation. An autosomal recessive primary immunodeficiency associated with classic Kaposi sarcoma of childhood and poor T-cell recall immune responses due to complete functional OX40 deficiency.

Immunogen: Recombinant protein within Human OX40/TNFRSF4 aa 29-214 (HA210892).

Positive control: Recombinant Human OX40/TNFRSF4 protein (HA210892).

Subcellular location: Membrane.

Database links: SwissProt: P43489 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH10-18] to Human OX40/TNFRSF4 antibody (Detector) (HA723188) and Recombinant Human OX40/TNFRSF4 protein (HA210892) as the standard. The reference range value is 31.25-8,000 pg/ml.

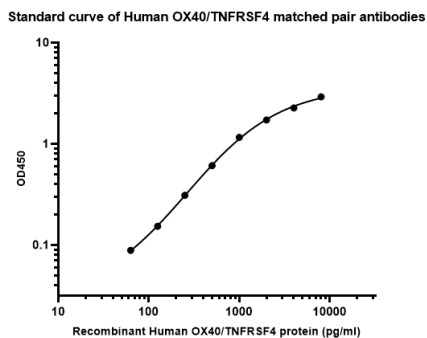
Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

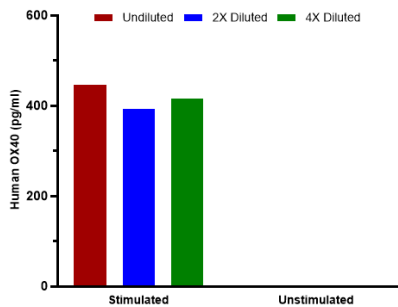
Images

Fig1: Sandwich ELISA analysis of Human OX40/TNFRSF4 matched pair antibodies



Elisa assay was performed by coating wells of a 96-well plate with 50 μ l per well of capture antibody (HA723187) diluted in carbonate/bicarbonate buffer, at a concentration of 5 μ g/mL overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150 μ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human OX40/TNFRSF4 protein (HA210892) starting from 8,000 pg/ml to 0 pg/ml and detect antibody (HA723188, Biotin, 0.2 μ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 50 μ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

Fig2: Interpolated concentrations of native OX40 in human PBMC cell culture supernatant.



PBMC cells were stimulated with 10 μ g/ml PHA-M or vehicle control and incubated for 5 days. The concentrations of OX40 measured, interpolated from the OX40 standard curve and corrected for sample dilution. Undiluted samples are as follows: unstimulated 100% and stimulated 100%. The mean OX40 concentration was determined to be 418.6 pg/ml in PHA-M stimulated PBMC cell culture supernatant and undetectable in the unstimulated PBMC control.

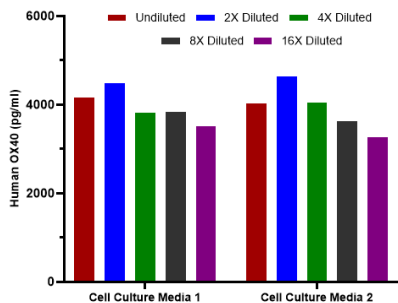


Fig3: Interpolated concentrations of spiked OX40 in human cell culture media samples.

The concentrations of OX40 were interpolated from the OX40 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 1 50%, cell culture media 2 50%.

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Baum P.R., Gayle R.B. III, Ramsdell F., Srinivasan S., Sorensen R.A., Watson M.L., Seldin M.F., Clifford K.N., Grabstein K., Alderson M.R. Identification of OX40 ligand and preliminary characterization of its activities on OX40 receptor. *Circ. Shock* 44:30-34 (1994)
2. Tang H., Serada S., Kawabata A., Ota M., Hayashi E., Naka T., Yamanishi K., Mori Y. CD134 is a cellular receptor specific for human herpesvirus-6B entry. *Proc. Natl. Acad. Sci. U.S.A.* 110:9096-9099 (2013)

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