

Human OX40/CD134 Sandwich ELISA Kit Datasheet

For the quantitative detection of human OX40 in serum and plasma.

General Information

Catalogue Number	KE00209
Product Name	Human OX40/CD134 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	7293
SwissProt	P43489

Kit Components & Storage

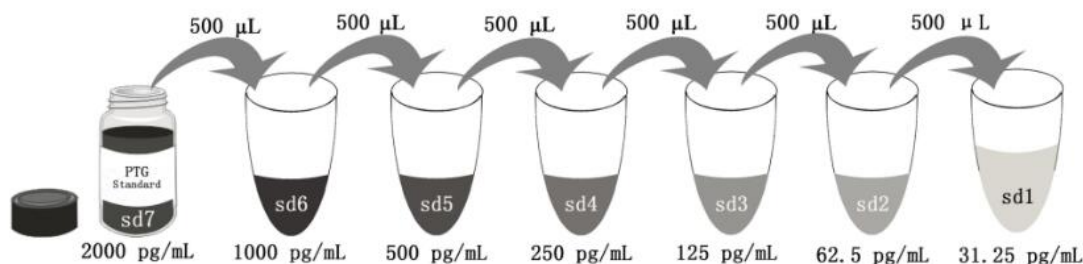
Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 5 - 30 mL/bottle. For tissue lysates.	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 5 is for protein protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 5 in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 5	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00209 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The OX40 ELISA kit is to be used to detect and quantify protein levels of endogenous OX40. The assay recognizes human OX40. An antibody specific for OX40 has been pre-coated onto the microwells. The OX40 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for OX40 is added to detect the captured OX40 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

OX40 (CD134) is a member of the TNFR-superfamily of receptors. Predominantly expressed on activated T cells, OX40 is activated by its cognate ligand OX40L (CD134L) and functions as a T cell co-stimulatory molecule. OX40-OX40L interactions have been proposed as a potential therapeutic target for treating autoimmunity. OX40 can interact with TRAF2, TRAF3, and TRAF5. In addition to a membrane bound form, OX40 also exists as a soluble form, released by activated lymphocytes.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for serum and plasma.

Safety Notes

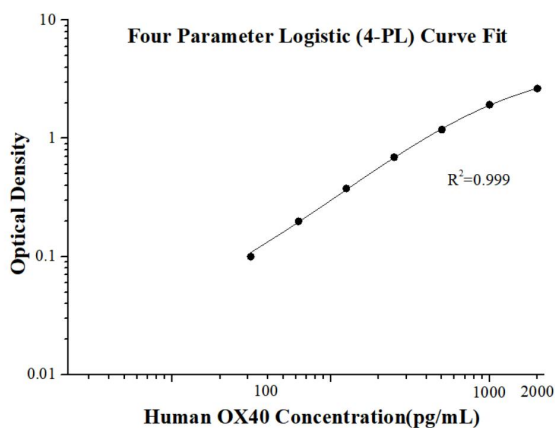
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.020 0.017	0.018	-
31.25	0.126 0.111	0.118	0.10
62.5	0.237 0.197	0.217	0.198
125	0.427 0.364	0.395	0.377
250	0.790 0.632	0.711	0.693
500	1.237 1.167	1.202	1.184
1000	2.052 1.836	1.944	1.926
2000	2.722 2.607	2.665	2.646

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	79.1	2.8	3.6	1	24	68.9	5.8	8.4
2	20	290.5	9.1	3.1	2	24	278.3	25.4	9.1
3	20	1,270.6	67.0	5.3	3	24	1,144.6	59.9	5.2

Recovery

The recovery of OX40 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Range (%)	Average% of Expected
Human serum	1:2	85-110	100
	1:4	84-102	96
Human plasma	1:2	101-118	111
	1:4	98-110	104

Sample Values

Serum and plasma samples from healthy volunteers were evaluated for OX40 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=24)	37.9-483	142.4
Human plasma (n=16)	22.9-518.7	96.5

Sensitivity

The minimum detectable dose of human OX40 is 5.8 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of OX40 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

Sample Type		Range (%)	Average% of Expected
Human serum	1:2	82-93	97
	1:4	78-89	84
	1:8	81-89	84
	1:16	84-89	87
Human plasma	1:2	98-104	101
	1:4	84-98	91
	1:8	92-96	94

References

1. Mallett S, Fossum S, Barclay AN. Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes--a molecule related to nerve growth factor receptor. *EMBO J.* 1990;9(4):1063-1068.
 2. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB. *Mol Cell Biol.* 1998 Jan;18(1):558-65.
 3. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchiyama T. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-kappaB activation. *J Biol Chem.* 1998 Mar 6;273(10):5808-14.
 4. Webb GJ, Hirschfield GM, Lane PJ. OX40, OX40L and Autoimmunity: a Comprehensive Review. *Clin Rev Allergy Immunol.* 2016;50(3):312-332.
- Michel J, Langstein J, Hofstädter F, Schwarz H. A soluble form of CD137 (ILA/4-1BB), a member of the TNF receptor family, is released by activated lymphocytes and is detectable in sera of patients with rheumatoid arthritis. *Eur J Immunol.* 1998;28(1):290-295.