colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL2RA in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00140
Product Name	IL2RA ELISA Kit
Species cross-reactivity	Human IL2RA
Range (calibration Range)	78.1 - 5000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**3559** (Human)
SwissProt	**P01589** (Human)

kit components & storage

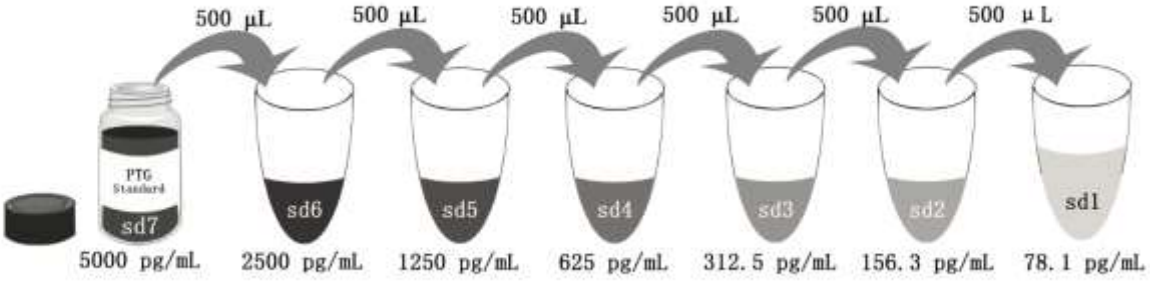
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 10000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection Antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 120 µL /vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 30 mL/bottle	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 1 is for standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add **2 mL** Sample Diluent PT 1 in standard. This reconstitution gives a stock solution of 5000 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 1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00140 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL2RA ELISA kit is to be used to detect and quantify protein levels of endogenous IL2RA. The assay recognizes human IL2RA. An antibody specific for IL2RA has been pre-coated onto the microwells. The IL2RA protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human IL2RA is added to detect the captured human IL2RA 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 450nm with the correction wavelength set at 630 nm.

background

Proliferation of T lymphocytes is triggered by the interaction of IL-2 with its specific receptor following T lymphocyte activation. The receptor for IL-2 has three forms, generated by different combinations of three different proteins, the alpha chain (IL-2R alpha), the beta chain (IL-2R beta), and the gamma chain (IL-2R gamma). IL-2R alpha (also known as CD25) is a type I transmembrane protein present on activated T cells, activated B cells, some thymocytes, myeloid precursors, and oligodendrocytes. Soluble IL-2R alpha has been isolated and determined to result from extracellular proteolysis. Soluble IL-2R alpha has been found in the plasma of patients with certain hematologic malignancies. Elevated plasma or serum concentration of soluble IL-2R alpha has also been reported as a biomarker of immune activation.

sample preparation

The serum, plasma, cell culture supernatants samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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safety notes

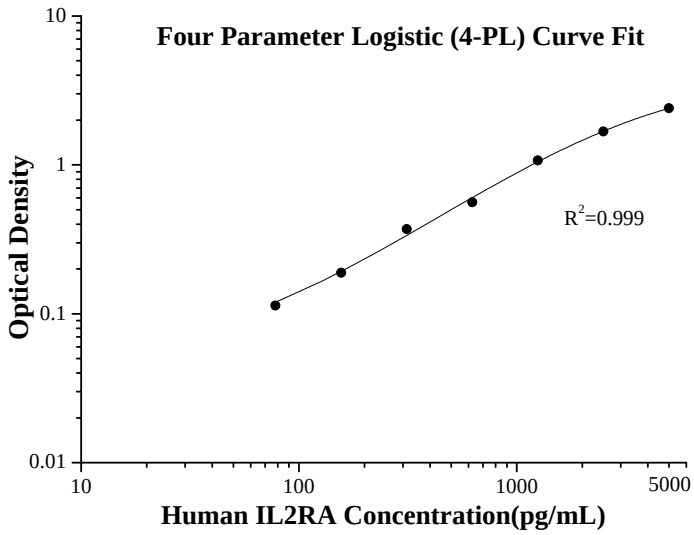
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

typical 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	0.084	0.081	-
	0.078		
78.1	0.200	0.195	0.114
	0.189		
156.2	0.265	0.270	0.189
	0.274		
312.5	0.456	0.452	0.371
	0.447		
625	0.609	0.642	0.561
	0.675		
1250	1.176	1.155	1.074
	1.133		
2500	1.741	1.759	1.678
	1.776		
5000	2.474	2.484	2.403
	2.494		

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.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	2,100.3	553.1	129.1	2,486.2	616.5	136.2
SD	208.2	48.6	11.1	237.1	48.4	12.4
CV%	9.9	8.8	8.6	9.5	7.9	9.1

recovery

The recovery of IL2RA spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	99	79-125
	1:4	85	70-98
Cell culture supernatants	1:2	107	102-113
	1:4	95	86-106

sample value

Serum and plasma samples were evaluated for IL2RA 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)
Healthy human serum (n=20)	278.7	10.2-1600.0
Healthy human plasma (n=18)	234.6	6.4-784.0
Liver cancer human serum (n=10)	1,059.8	405.6-1,922.4

Human peripheral blood leuocytes cells (1 x 10^6cells/mL) were cultured in DMEM supplemented with 8% fetal bonive serum, 5μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 1 day, 3 days and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IL2RA.

Condition	(pg/mL)
Unstimulated	-
Stimulated for 1d	373.9
Stimulated for 3d	4,245.2
Stimulated for 5d	12,558.4

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sensitivity

The minimum detectable dose of human IL2RA 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, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	113	107
	Range (%)	84-126	105-114
1:8	Average% of Expected	95	115
	Range (%)	81-107	108-123
1:16	Average% of Expected	89	122
	Range (%)	78-100	121-123

references

1. Minami Y, et al. The IL-2 receptor complex: its structure, function, and target genes. Annu Rev Immunol. 11:245-68 (1993).
2. Akin C, et al. Soluble stem cell factor receptor (CD117) and IL-2 receptor alpha chain (CD25) levels in the plasma of patients with mastocytosis: relationships to disease severity and bone marrow pathology. Blood. 96(4):1267-73 (2000).
3. Litzman J, et al. Chronic immune activation in common variable immunodeficiency (CVID) is associated with elevated serum levels of soluble CD14 and CD25 but not endotoxaemia. Clin Exp Immunol. 170(3):321-32 (2012).