Human IL-2 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-2 in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00017
Product Name	Human IL-2 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3558
SwissProt	P60568

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated(100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 1-df - 30 mL/bottle. For cell culture supernatants.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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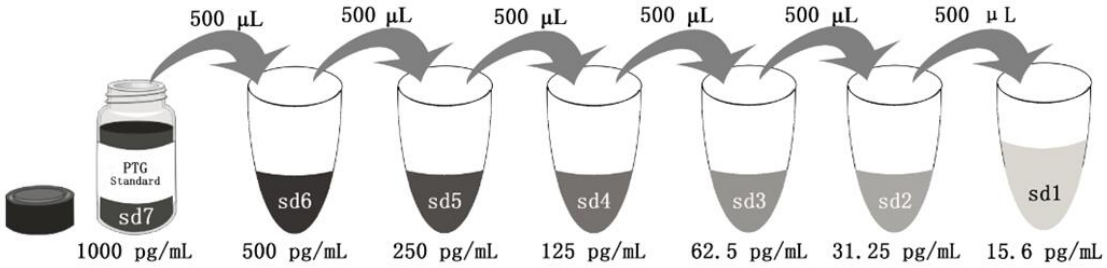
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard, serum and plasma samples.
Sample Diluent PT 1-df is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-ef or PT 1-df in standard. This reconstitution gives a stock solution of 1000 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-ef or PT 1-df	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00017 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-2 ELISA kit is to be used to detect and quantify protein levels of endogenous IL-2. The assay recognizes human IL-2. An antibody specific for IL-2 has been pre-coated onto the microwells. The IL-2 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL-2 is added to detect the captured IL-2 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

Interleukin-2 (IL-2) is a four-helix bundle, type I cytokine that functions as a growth factor for a wide range of leukocytes. In the immune system, IL-2 is essential for immune homeostasis, normal T regulatory cell function, and self-tolerance. It regulates immune cell homeostasis and has been used to treat a range of disorders including cancer and autoimmune disease. IL-2 signals through heterodimerization of theIL-2R β and IL-2R γ receptor subunits.

Sample Preparation

The serum or plasma 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.				

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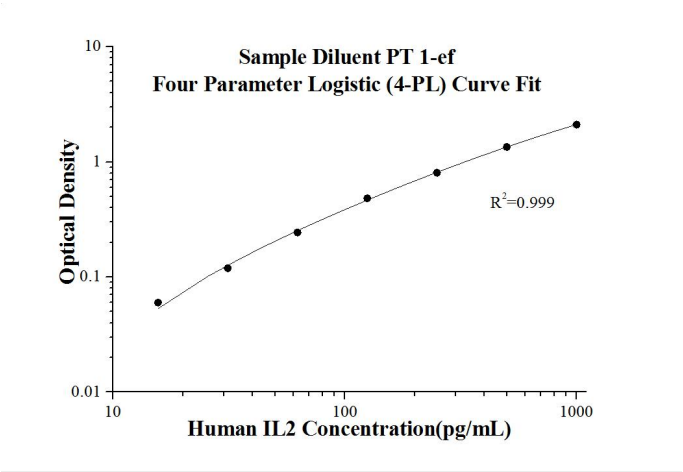
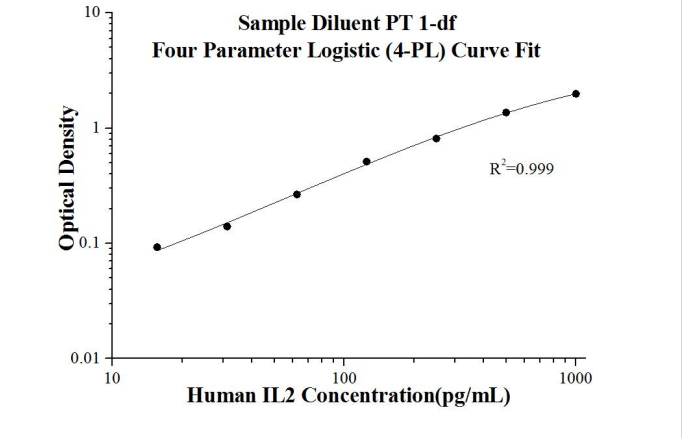
Example data

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	0.033 0.033	0.033	—
15.63	0.093 0.093	0.093	0.06
31.25	0.151 0.153	0.152	0.119
62.5	0.288 0.266	0.277	0.244
125	0.511 0.519	0.515	0.482
250	0.84 0.835	0.8375	0.8045
500	1.356 1.407	1.3815	1.3485
1000	2.096 2.185	2.1405	2.1075



(pg/mL)	O.D	Average	Corrected
0	0.03 0.029	0.0295	-
15.6	0.143 0.101	0.122	0.0925
31.25	0.19 0.149	0.1695	0.14
62.5	0.344 0.246	0.295	0.2655
125	0.604 0.478	0.541	0.5115
250	0.956 0.725	0.8405	0.811
500	1.467 1.319	1.393	1.3635
1000	2.026 1.997	2.0115	1.982

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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				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	1109.7	35.8	3.2
2	20	339.3	15.7	4.6
3	20	70.8	2.0	2.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1370.3	126.4	9.2
2	24	420.6	32.5	7.7
3	24	79.9	6.5	8.1

Recovery

The recovery of IL-2 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 plasma	1:2	88	81-96
	1:4	95	84-107
Cell culture supernatants	1:2	118	116-121
	1:4	107	103-109

Sample Values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL-2 in this assay. All samples measured less than the lowest standard, 15.6 pg/mL. No medical histories were available for the donors used in this study.

Sensitivity

The minimum detectable dose of human IL-2 is 3.6 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of IL-2 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

		Human plasma (Sample Diuent PT1-ef)	Cell culture supernatants (Sample Diuent PT1-df)
1:2	Average% of Expected	83	120
	Range (%)	80-86	115-124
1:4	Average% of Expected	97	117
	Range (%)	93-101	111-121
1:8	Average% of Expected	106	110
	Range (%)	97-114	99-122
1:16	Average% of Expected	102	108
	Range (%)	98-106	102-114

Calibration

This immunoassay is calibrated against a highly purified E. coli-expressed recombinant human IL-2 produced at Proteintech Systems.

NIBSC/WHO International standard for IL-2(86/500), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard(86/500) parallels the Proteintech standard curve. To convert sample values obtained with the Human IL-2 ELISA kit to approximate NIBSC (86/500) units, use the equation below.

NIBSC (86/500) approximate value (IU/mL) = 0.0170 x Proteintech Human IL-2 value (pg/mL)

References

1. Spangler JB. et al. (2015). Immunity. 42: 815-25.
2. Rosenberg SA. et al. (2014). J Immunol. 192: 5451-8.
3. Nelson BH. et al. (2004). J Immunol. 172: 3983-8.
4. Turka LA. et al. (2008). Front Biosci. 13: 1440-6.

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