

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL4 in serum, plasma, cell culture supernatants.

general information

Catalogue Number	KE00016
Product Name	IL4 ELISA Kit
Species cross-reactivity	Human IL4
Range (calibration Range)	15.6 - 1000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	3565 (Human)
SwissProt	P05112 (Human)

kit components & storage

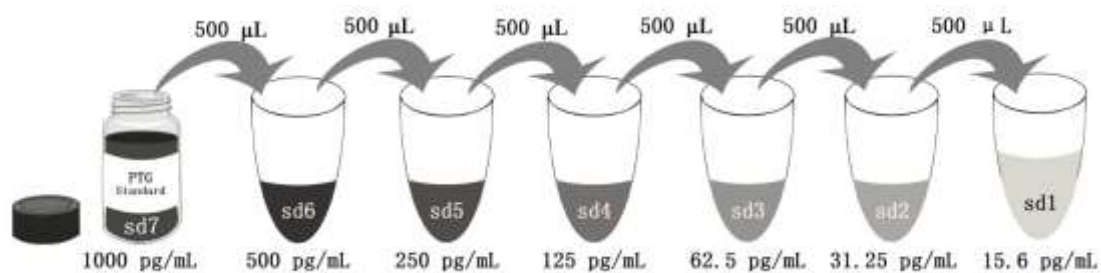
Microplate - antibody coated 96-well Microplate (8 wells × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 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-ef is for standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP antibody.

*Add 1 mL Sample Diluent PT 1-ef 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	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00016 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL4 ELISA kit is to be used to detect and quantify protein levels of endogenous IL4. The assay recognizes human IL4. An antibody specific for IL4 has been pre-coated onto the microwells. The IL4 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL4 is added to detect the captured IL4 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

Interleukin-4 (IL-4), a member of the α -helical cytokine family, is produced by activated CD4 + T cells, basophils, and mast cells. It promotes the proliferation and differentiation of antigen presenting cells. IL4 also plays a pivotal role in antibody isotype switching and stimulates the production of IgE. This cytokine has been applied in the treatment of autoimmune disorder like multiple myeloma, cancer, psoriasis, and arthritis. IL4 has also been extensively applied to inhibit detrimental effect of Th1. It may promote the growth of epithelial tumors by mediating increased proliferation and survival.

sample preparation

The serum, plasma or 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.

safety notes

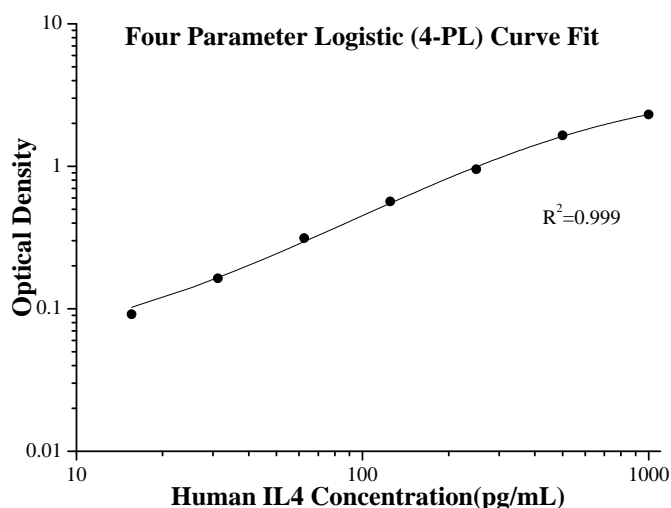
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.041	0.038	—
	0.035		
15.6	0.123	0.1295	0.0915
	0.136		
31.25	0.204	0.2015	0.1635
	0.199		
62.5	0.333	0.351	0.313
	0.369		
125	0.603	0.604	0.566
	0.605		
250	0.992	0.992	0.954
	0.992		
500	1.674	1.6865	1.6485
	1.699		
1000	2.306	2.342	2.304
	2.378		

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	777.0	225.5	43.3	703.8	212.2	42.4
SD	45.1	6.4	2.4	33.8	13.3	1.9
CV%	5.8	2.8	5.5	4.8	6.2	4.5

recovery

The recovery of IL4 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	1:4	102	86-113
	1:8	104	88-116
Cell culture supernatants	1:2	101	85-105
	1:4	88	83-95

sample value

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL4 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 IL4 is 2.2 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 IL4 in various matrices and diluted with the appropriate **Sample Diluent PT 1-ef** to produce samples with values within the dynamic range of the assay. (The serum and plasma samples were initially diluted 1:1)

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	100	114
	Range (%)	93-107	101-124
1:4	Average% of Expected	106	100
	Range (%)	89-125	97-105
1:8	Average% of Expected	105	96
	Range (%)	93-122	92-100
1:16	Average% of Expected	104	98
	Range (%)	100-111	95-100

calibration

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL4 produced at Proteintech Systems.

The NIBSC/WHO International Standard for IL4 (88/656), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard (88/656) parallels the Proteintech standard curve. To convert sample values obtained with the Human IL4 ELISA kit to approximate NIBSC 88-656 units, use the equation below.

NIBSC (88-656) approximate value (IU/mL) = 0.0189x Proteintech Human IL-4 value (pg/mL)

references

1. Dhanda SK. et al.(2013) Clin Dev Immunol. doi: 10.1155/2013/263952.
2. Müller-Hermelink N. et al. (2008) Cancer Cell.13: 507-18.
3. Leberman DA. et al. (1988) 168: 853-62.
4. provided by RefSeq, Jul 2008.