colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL10 in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00012
Product Name	IL10 ELISA Kit
Species cross-reactivity	Human IL10
Range (calibration Range)	7.8 - 500 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	3586 (Human)
SwissProt	P22301 (Human)

kit components & storage

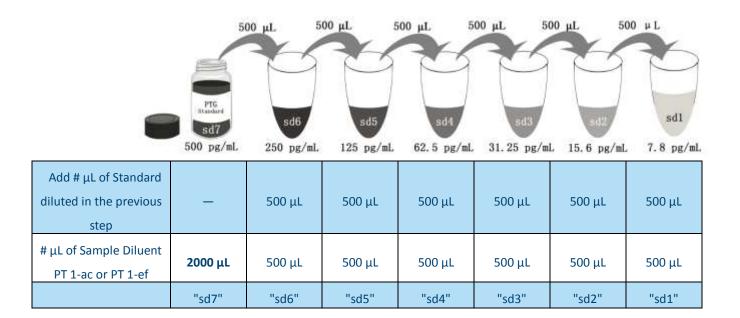|		
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard -1000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 μL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ac - 30 mL/bottle; For serum and plasma samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle; For cell culture supernatants	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-ac is for standard, serum and plasma samples.

Sample Diluent PT 1-ef is for standard and cell culture supernatants.

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

*Add 2 mL Sample Diluent PT 1-ac or PT 1-ef in standard. This reconstitution gives a stock solution of 500 pg/mL.

	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	500 pg/mL	250 pg/mL	125 pg/mL	62.5 pg/mL	31.25 pg/mL	15.6 pg/mL	7.8 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-ac or PT 1-ef	2000 μL	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00012 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL10 ELISA kit is to be used to detect and quantify protein levels of endogenous IL10. The assay recognizes human IL10. An antibody specific for IL10 has been pre-coated onto the microwells. The IL10 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IL10 is added to detect the captured IL10 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody 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 (IL)-10 is an anti-inflammatory cytokine, produced by T helper (Th) cells, macrophages, monocytes, and B cells, that plays a crucial role in preventing inflammatory and autoimmune pathologies. It downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway. IL10, along with its receptors, describes an important role in pathogenesis of various diseases, including infectious, inflammatory, autoimmune diseases. IL10 mutations are associated with an increased susceptibility to HIV-1 infection and rheumatoid arthritis.

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.

safety notes

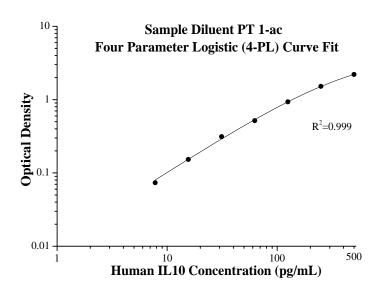This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
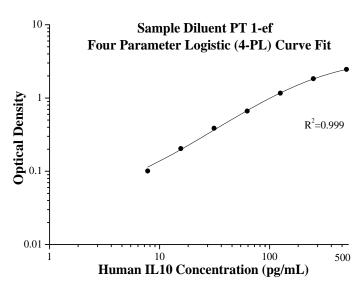
USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

ptglab.com

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.

Sample Diluent PT 1-ac
Four Parameter Logistic (4-PL) Curve Fit

$R^2 = 0.999$

(pg/mL)	O.D	Average	Corrected
0	0.103	0.1025	—
	0.102		
7.8	0.177	0.176	0.0735
	0.175		
15.6	0.256	0.255	0.1525
	0.254		
31.3	0.408	0.4155	0.313
	0.423		
62.5	0.632	0.6195	0.517
	0.607		
125	1.034	1.034	0.9315
	1.034		
250	1.681	1.618	1.5155
	1.555		
500	2.318	2.312	2.2095
	2.306		

Sample Diluent PT 1-ef
Four Parameter Logistic (4-PL) Curve Fit

$R^2=0.999$

(pg/mL)	O.D	Average	Corrected
0	0.106	0.1075	—
	0.109		
7.8	0.183	0.1845	0.077
	0.186		
15.6	0.247	0.243	0.1355
	0.239		
31.3	0.434	0.4065	0.299
	0.379		
62.5	0.622	0.6195	0.512
	0.617		
125	0.999	0.9905	0.883
	0.982		
250	1.561	1.6	1.4925
	1.639		
500	1.977	2.026	1.9185
	2.075		

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	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	38.9	133.4	484.9	39.9	137.5	527.0
SD	1.7	7.0	17.1	2.4	7.8	39.7
CV%	4.3	5.2	3.5	6.1	5.7	7.5

recovery

The recovery of IL10 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	95	80-114
	1:4	104	86-127
Cell culture supernatants	1:2	102	87-114
	1:4	99	88-107

sample values

Twenty serum and plasma samples from healthy volunteers were evaluated for human IL10 in this assay. Sixteen samples measured less than the lowest standard, 7.8 pg/mL. Four samples measured between 114 and 1,580 pg/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human IL10 is 0.5 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 IL10 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma (Sample Diluent PT 1-ac)	Cell culture supernatants (Sample Diluent PT 1-ef)
1:2	Average% of Expected	91	88
	Range (%)	90-92	86-88
1:4	Average% of Expected	97	86
	Range (%)	93-101	79-97
1:8	Average% of Expected	99	88
	Range (%)	97-99	80-96
1:16	Average% of Expected	104	91
	Range (%)	102-107	85-97

calibration

This immunoassay is calibrated against highly purified *E. coli*-expressed 160 amino acid form of recombinant human IL10 produced at Proteintech Systems.
The NIBSC/WHO International Standard for IL10 (93/722), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard (93/722) parallels the Proteintech standard curve. To convert sample values obtained with the Human IL10 ELISA kit to approximate NIBSC 93/722 units, use the equation below.
NIBSC (93/722) approximate value (IU/mL)=0.234x Proteintech Human IL10 value (pg/mL)

references

1. Mosmann TR. et al. (1994). Adv Immunol. 56: 1-26.

2. Kühn R. et al. (1993). Cell. 75: 263-74.

3. Turner DM. et al. (1997). Eur J Immunogenet. 24: 1-8.

4. Peng H. et al. (2013). Clin Rheumatol. 2013 May 25.

5. Schall TJ. et al. (1990). Nature. 347: 669–671.

6. Cai G. et al. (1999). Eur J Immunol 29: 2658–2665.

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