

## colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL18 in serum and plasma.

### general information

Catalogue Number	KE00025
Product Name	IL18 ELISA Kit
Species cross-reactivity	Human IL18
Range (calibration Range)	15.6 - 1000 pg/mL
Tested applications	Quantification ELISA

### database links

Entrez Gene	3606 (Human)
SwissProt	Q14116 (Human)

### kit components & storage

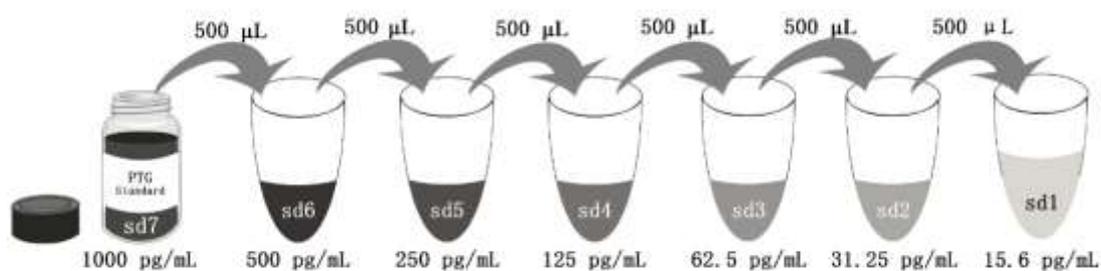
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 2000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-e - 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-e is for standard and samples.

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

\*Add **2 mL** Sample Diluent PT 1-e in standard. This reconstitution gives a stock solution of 1000 pg/mL.



Add # $\mu\text{L}$ of Standard diluted in the previous step	—	500 $\mu\text{L}$					
# $\mu\text{L}$ of Sample Diluent PT 1-e	2000 $\mu\text{L}$	500 $\mu\text{L}$					
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## product description

KE00025 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL18 ELISA kit is to be used to detect and quantify protein levels of endogenous Pre-IL18 and mature IL18. The assay recognizes human IL18. An antibody specific for IL18 has been pre-coated onto the microwells. The IL18 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IL18 is added to detect the captured IL18 protein. For signal development, horseradish peroxidase (HRP)-conjugated Anti-mouse 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

IL18, also named as IGIF and IL1F4, belongs to the IL1 family. It augments natural killer cell activity in spleen cells and stimulates interferon gamma production in T-helper type I cells. The IL18 cytokine increased expression of vascular cell adhesion molecule-1 (VCAM1) and the adherence of melanoma cells. IL18 plays a major role in atherosclerotic plaque destabilization leading to acute ischemic syndromes. IL18 negatively regulate NAFLD/NASH progression, as well as multiple aspects of metabolic syndrome via modulation of the gut microbiota. This kit is used to quantify Pre-IL18 and mature IL18 level in vivo.

## 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.

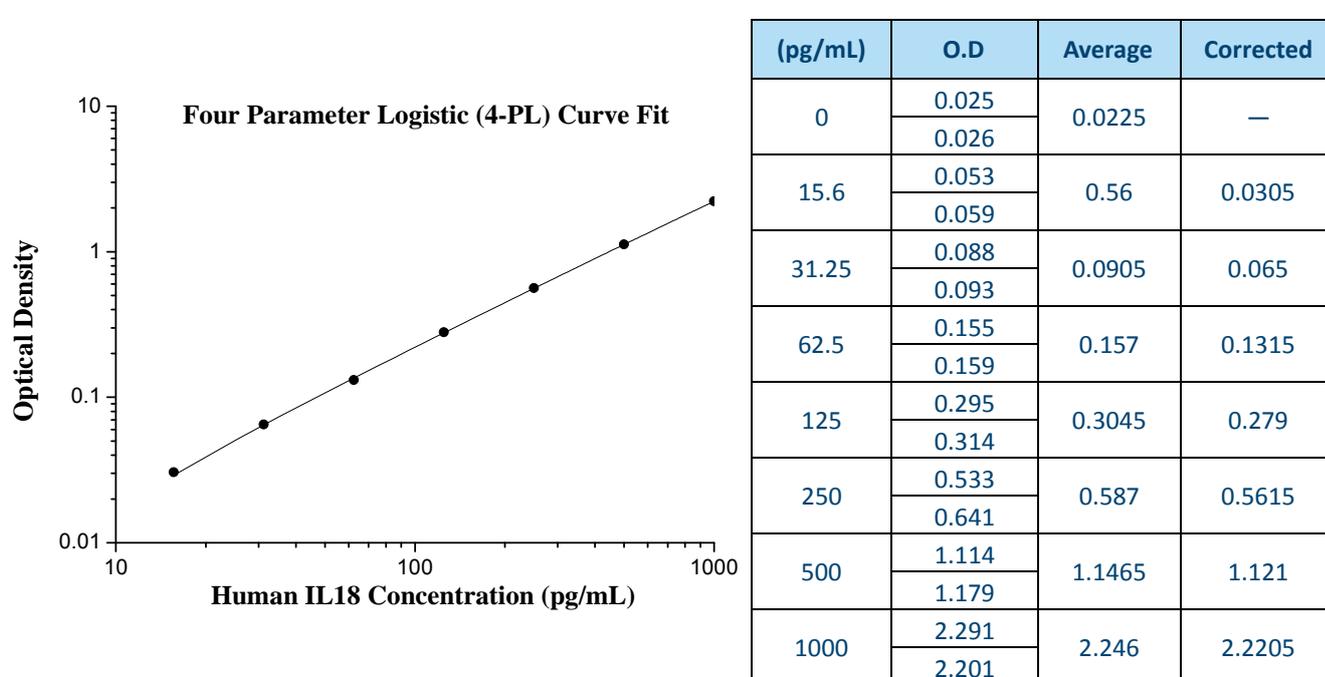
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 $\mu$ L	<b>120 min</b>	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 $\mu$ L	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 $\mu$ L	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 $\mu$ L	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 $\mu$ L	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.



## 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)	512.7	265.8	89.9	495.5	258.4	90.9
SD	18.4	9.9	5.5	20.6	13.3	4.2
CV%	3.6	3.7	6.1	4.2	5.2	4.7

## recovery

The recovery of IL18 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 87%, ranging from 77%-110%.

## sample values

Sixty-six serum and plasma samples from healthy volunteers were evaluated for human IL18 in this assay. Fifty-eight samples measured less than the lowest standard, 15.6 pg/mL. Eight samples measured between 16 and 26 pg/mL. No medical histories were available for the donors used in this study.

## sensitivity

The minimum detectable dose of human IL18 is 1.5 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 IL18 in human plasma and diluted with the appropriate **Sample Diluent PT 1-e** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Human plasma
1:2	Average% of Expected	99
	Range (%)	92-110
1:4	Average% of Expected	98
	Range (%)	92-108
1:8	Average% of Expected	106
	Range (%)	95-127
1:16	Average% of Expected	108
	Range (%)	95-130

## calibration

This immunoassay is calibrated against highly purified *E. coli*-expressed 193 amino acid form of recombinant human IL18 produced at Proteintech Systems.

The NIBSC/WHO International standard for IL18 (03/200), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard (03/200) parallels the Proteintech standard curve. To convert sample values obtained with the Human IL18 ELISA kit to approximate NIBSC 03/200 units, use the equation below.

NIBSC (03/200) approximate value (IU/mL)=1.488 x Proteintech Human Pre-IL18 value (pg/mL)

## references

1. Vidal-Vanaclocha F., *et al*, IL-18 regulates IL-1-beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc. Nat. Acad. Sci.* 97: 734-739, 2000.
2. Henao-Mejia J., *et al*, Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 482: 179-185, 2012.
3. Mallat Z., *et al*. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation* 104: 1598-1603, 2001.