

## Human IL-22 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL22 in serum, plasma and cell culture supernatants.

### General Information

Catalogue Number	KE00008
Product Name	Human IL-22 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	50616
SwissProt	Q9GZX6

### Kit Components & Storage

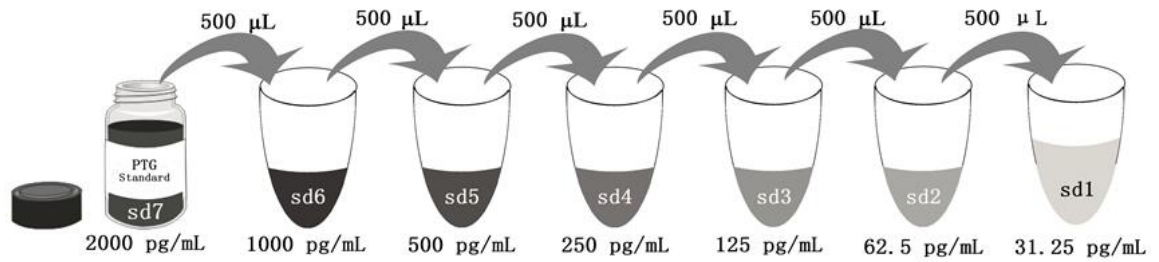
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µ L/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 4-ef - 30 mL/bottle	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	

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 4-ef is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 2 mL Sample Diluent PT 4-ef in protein standard. This reconstitution gives a stock solution of 2000 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 4-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00008 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL22 ELISA kit is to be used to detect and quantify protein levels of endogenous IL22. The assay recognizes human IL22. An antibody specific for IL22 has been pre-coated onto the microwells. The IL22 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL22 is added to detect the captured IL22 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 22 (IL22) is a member of the human type I interferon family, which includes IL10. IL22 has the potential to interact with IL10 because it binds to the IL-10R2c chain with IL22R1 in its receptor complex. Binding can be blocked by the soluble receptor, IL22 binding protein (IL22BP). Although known as a Th17 cytokine, IL22 is also expressed by a wide range of immune cells, including NK T,  $\gamma \delta$  T, and NK cells. IL22 can contribute to immune disease through the stimulation of inflammatory responses, S100s and defensins. IL22 also promotes hepatocyte survival in the liver and epithelial cells in the lung and gut similar to IL10.

## 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

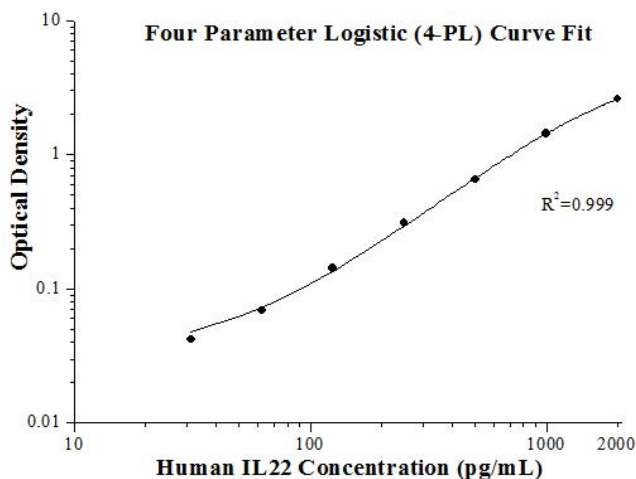
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 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.				

## 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	0.038 0.047	0.043	—
31.25	0.084 0.085	0.085	0.042
62.5	0.118 0.104	0.111	0.068
125	0.173 0.195	0.184	0.141
250	0.350 0.353	0.352	0.309
500	0.698 0.694	0.696	0.653
1000	1.456 1.517	1.487	1.444
2000	2.646 2.663	2.655	2.612

## 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	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	2035.3	70.9	3.5	1	24	2174.7	155.3	7.1
2	20	539.2	26.4	4.9	2	24	577.5	46.8	8.1
3	20	54.8	3.6	6.5	3	24	59.7	4.7	7.9

## Recovery

The recovery of IL22 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	115	108-119
	1:4	111	109-121
Cell culture supernatants	1:2	117	116-119
	1:4	110	101-119

## Sample Values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL22 in this assay. All samples measured between 172 pg/mL and 1462 pg/mL. No medical histories were available for the donors used in this study.

THP-1 cells ( $3 \times 10^6$  cells/mL) were cultured in RPMI with 10% fetal bovine serum, 50  $\mu$  M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$  g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 50 ug/mL LPS for 1 and 5 days. Aliquots of the cell culture supernatants were removed and assayed for levels of natural IL22.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	337	259
Stimulated	417	270

## Sensitivity

The minimum detectable dose of human IL22 is 14.2 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 IL22 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	100	114
	Range (%)	93-107	102-117
1:4	Average% of Expected	110	103
	Range (%)	101-115	89-104
1:8	Average% of Expected	107	102
	Range (%)	103-112	90-105
1:16	Average% of Expected	94	112
	Range (%)	88-101	102-115

## References

1. Pestka S. et al. (2004) Annu Rev Immunol. 22: 929-79.
2. Wolk K. et al. (2002) J Immunol 168(11): 5397-5402.
3. Zheng Y et al. (2007) Nature 445: 648-651.
4. Zheng Y et al. (2008) Nat Med 14: 282-289.
5. Xie MH, et al. (2000) J Biol Chem. 275: 31335-9.
6. Jones BC, et al. (2008) 16:1333-44.