Human IL-1 beta Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-1 beta in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00021
Product Name	Human IL-1 beta Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	3.9-250 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3553
SwissProt	P01584

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 - 500 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 3-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	

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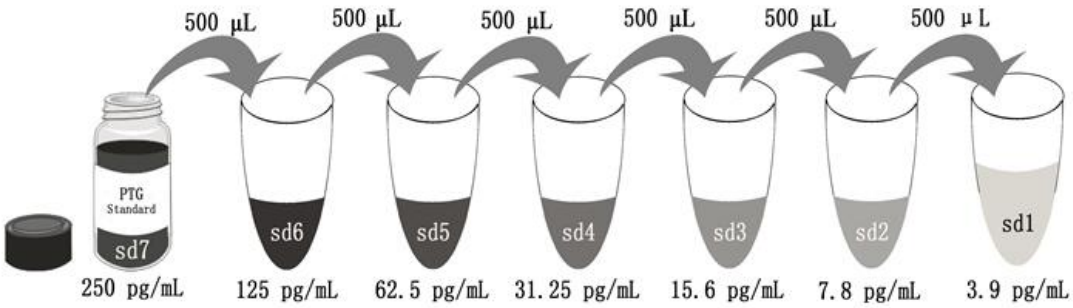
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 3-ef in protein standard. This reconstitution gives a stock solution of 250 pg/mL.



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 3-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00021 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-1 beta ELISA kit is to be used to detect and quantify protein levels of endogenous IL-1 beta. The assay recognizes human IL-1 beta. An antibody specific for IL-1 beta has been pre-coated onto the microwells. The IL-1 beta protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL-1 beta is added to detect the captured IL-1 beta protein. For signal development, Sterptravidin-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-1 is a pro-inflammatory cytokine with multiple biological effects. The IL-1 gene family encodes three proteins: IL-1 α , IL-1 beta and their naturally occurring inhibitor IL-1RN. Interleukin 1 beta (IL-1 beta), mainly produced by blood monocytes and tissue macrophages, has been implicated in mediating both acute and chronic inflammation. IL-1 beta is known to be involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis. IL-1 beta is emerging as a key mediator of carcinogenesis that characterizes host-environment interactions.

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

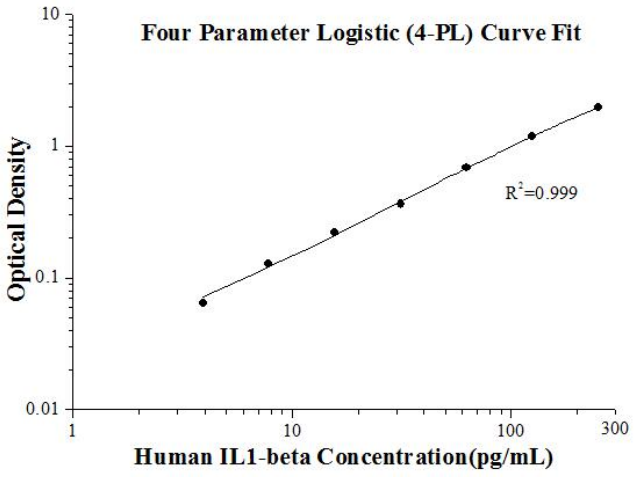
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

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	0.025 0.043	0.034	-
3.91	0.097 0.099	0.098	0.064
7.81	0.175 0.149	0.162	0.128
15.63	0.284 0.233	0.257	0.223
31.25	0.407 0.385	0.396	0.362
62.5	0.738 0.698	0.718	0.684
125	1.255 1.178	1.217	1.1825
250	2.072 1.927	2.000	1.966

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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	276.5	6.8	2.4
2	20	68.9	2.2	3.2
3	20	16.2	0.5	2.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	294.5	6.9	2.3
2	24	73.0	1.9	2.7
3	24	15.7	1.1	7.2

Recovery

The recovery of IL-1 beta 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	93	91-95
	1:4	109	100-117
Cell culture supernatants	1:2	104	88-124
	1:4	102	83-119

Sample Values

Twenty serum and plasma samples from healthy volunteers were evaluated for human IL-1 beta 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.

THP-1 cells (3 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 μM β-mercaptoethanol , 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured stimulated with 50 ug/mL LPS for 5 days. The cell culture supernatants were assayed for levels of IL-1 beta and measured 39 pg/mL.

Sensitivity

The minimum detectable dose of human IL-1 beta 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.

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Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of IL-1 beta 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	112	111
	Range (%)	102-115	110-112
1:4	Average% of Expected	114	116
	Range (%)	109-125	112-119
1:8	Average% of Expected	113	117
	Range (%)	102-125	114-119
1:16	Average% of Expected	106	112
	Range (%)	100-108	107-116

References

1. Dinarello CA. et al. (1996). Blood. 87: 2095-147.
2. Bird S. et al. (2002). Cytokine Growth Factor Rev. 13: 483-502.
3. Xu J. et al. (2013). PLoS One. 21;8(5):e63654.
4. McCarty S. (2014). Cardiol Rev. 22: 176-81.

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