Human IL-8 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-8 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00006
Product Name	Human IL-8 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3576
SwissProt	P10145

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 - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 4-e - 30 mL/bottle. For human serum and plasma samples	1 bottle	
Sample Diluent PT 4-ef - 30 mL/bottle. For cell culture supernatants.	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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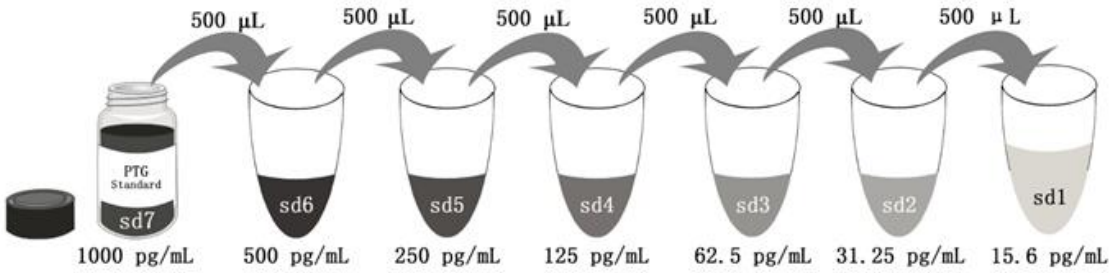
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4-e is for protein standard, serum and plasma samples.
Sample Diluent PT 4-ef is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 4-e or PT 4-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 4-e or 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

KE00006 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL8 ELISA kit is to be used to detect and quantify protein levels of endogenous IL8. The assay recognizes human IL8. An antibody specific for IL8 has been pre-coated onto the microwells. The IL8 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IL8 is added to detect the captured IL8 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 450 nm with the correction wavelength set at 630 nm.

Background

Interleukin 8 (IL8), also known as CXCL8, which is a member of the CXC chemokine family. This chemokine is secreted by a variety of cell types including monocyte/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, and various tumor cell lines in response to inflammatory stimuli. IL8 has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL8 also induces phagocytosis once they have arrived. This gene is believed to play a role in the pathogenesis of bronchiolitis, a common respiratory tract disease caused by viral infection. IL8 is also known to be a potent promoter of angiogenesis. IL8 has been associated with tumor angiogenesis, metastasis, and poor prognosis in breast cancer. IL-8 may present a novel therapeutic target for estrogen driven breast carcinogenesis and tumor progression.

Sample Preparation

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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.				

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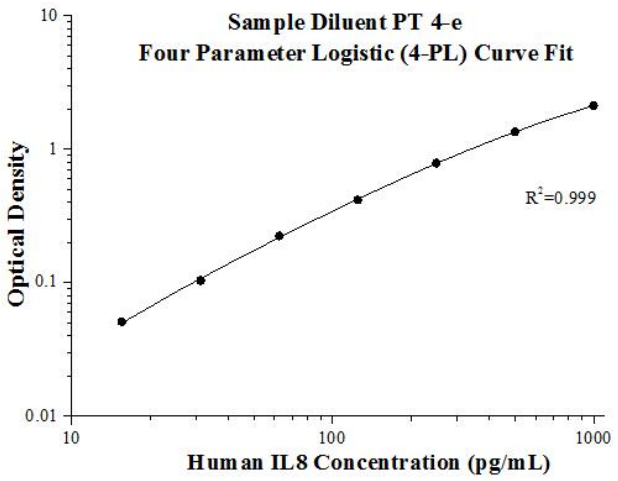
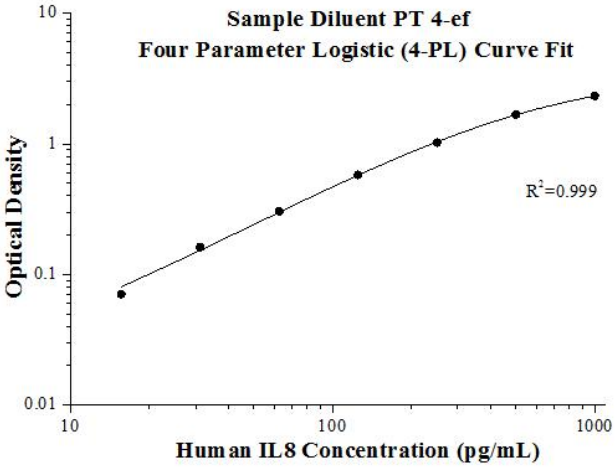
Example data

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	0.047 0.045	0.046	—
15.6	0.1 0.094	0.097	0.051
31.25	0.156 0.144	0.15	0.104
62.5	0.276 0.266	0.271	0.225
125	0.465 0.462	0.463	0.4175
250	0.837 0.829	0.833	0.787
500	1.431 1.36	1.395	1.3495
1000	2.215 2.112	2.163	2.1175



(pg/ml)	O.D	Average	Corrected
0	0.051 0.055	0.053	—
15.6	0.132 0.115	0.123	0.07
31.25	0.222 0.207	0.214	0.161
62.5	0.36 0.354	0.357	0.304
125	0.655 0.606	0.63	0.577
250	1.065 1.082	1.073	1.02
500	1.765 1.688	1.726	1.673
1000	2.383 2.367	2.375	2.32

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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	127.3	5.3	4.1
2	20	411.6	23.7	5.8
3	20	804.4	44.7	5.6

Inter-assay Precision				
Sample	n	Mean (pg/ml)	SD	CV%
1	24	123.7	4.8	3.9
2	24	420.9	17.1	4.1
3	24	788.9	35.8	4.5

Recovery

The recovery of IL8 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

Serum/Plasma-Samples from healthy volunteers were evaluated for human IL8 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human plasma (n=28)	87	25	ND-136

Sensitivity

The minimum detectable dose of human IL8 is 1.0 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 IL8 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 4-e)	Cell culture supernatants (Sample Diluent PT 4-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 99 amino acid form of recombinant human IL8 produced at Proteintech Systems.

The NIBSC/WHO International Standard for IL8 (89/520), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard (89/520) parallels the Proteintech standard curve. To convert sample values obtained with the Human IL8 ELISA kit to approximate NIBSC 89/520 units, use the equation below.

NIBSC (89/520) approximate value (IU/mL)=0.00048× Proteintech Human IL8 value (pg/mL)

References

1. Wolff B. et al., 1998. J Exp Med. 188: 1757-1762.
2. Utgaard JO. et al., 1998. J Exp Med. 188: 1751-1756.
3. Modi WS. Et al., 1990. Hum Genet. 84: 185-187.
4. "Entrez Gene: IL8 interleukin 8".
5. Bendrik C. et al. 2009. J Immunol. 182(1): 371-378.

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