

Human IL-3 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-3 concentrations in serum, plasma and cell culture supernatants .

General Information

Catalogue Number	KE00185
Product Name	Human IL-3 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	7.8 - 500 pg/mL, 15.6 - 1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3562
SwissProt	P08700

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 - 1000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle. For cell culture supernatants	1 bottle	
Sample Diluent PT 3B5 - 30 mL/bottle. For serum and plasma	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.

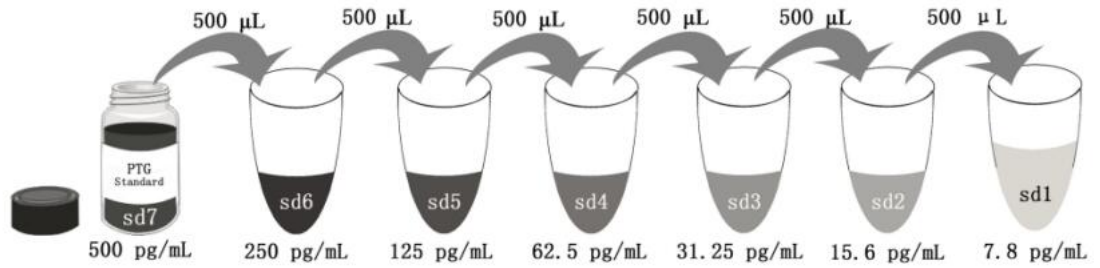
Sample Diluent PT 4B1 is for protein standard and cell culture supernatants.

Sample Diluent PT 3B5 is for protein standard, serum and plasma.

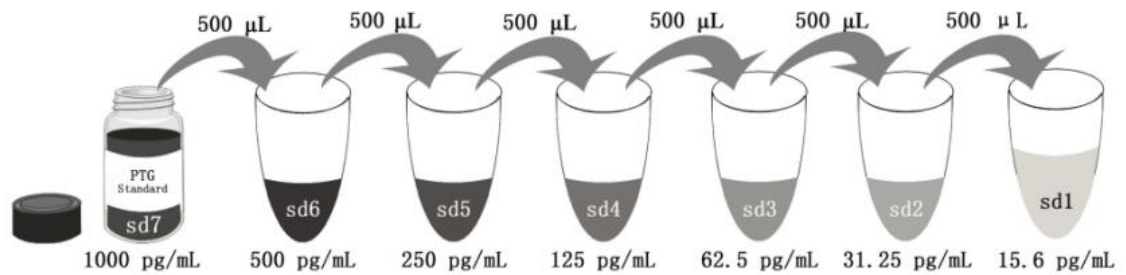
Detection Diluent is for Detection antibody.

*Add 2 mL Sample Diluent PT 4B1 in protein standard. This reconstitution gives a stock solution of 500 pg/mL.

*Add 1 mL Sample Diluent PT 3B5 in protein 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 4B1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"



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 3B5	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00185 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-3 ELISA kit is to be used to detect and quantify protein levels of endogenous IL-3. The assay recognizes human IL-3. An antibody specific for IL-3 has been pre-coated onto the microwells. The IL-3 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IL-3 is added to detect the captured IL-3 protein. For signal development, 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-3 (IL-3) is a multilineage hematopoietic growth factor that promotes the proliferation, differentiation and survival of early multilineage hematopoietic progenitors. In particular, this cytokine plays a key role in stimulating the proliferation and survival of myeloid precursors. It is involved in a variety of cell activities such as cell growth, differentiation and apoptosis. This cytokine has been shown to also possess neurotrophic activity, and it may be associated with neurologic disorders.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for cell culture supernatants.

Safety Notes

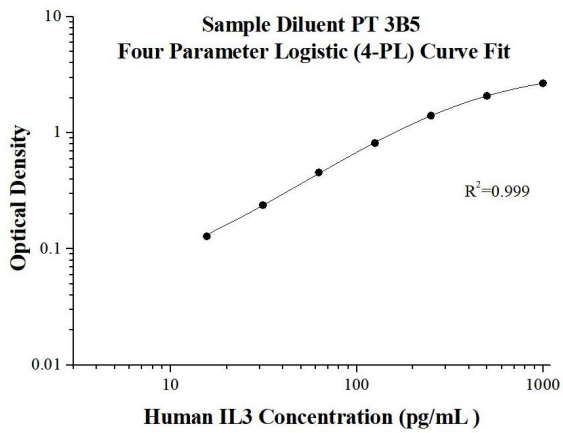
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

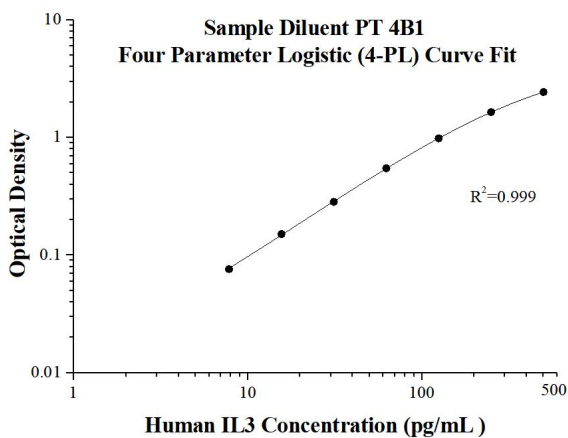
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Detection Antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	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	0.102 0.107	0.105	-
15.6	0.231 0.235	0.233	0.129
31.25	0.342 0.344	0.343	0.239
62.5	0.555 0.566	0.561	0.456
125	0.924 0.916	0.920	0.816
250	1.500 1.520	1.510	1.406
500	2.170 2.204	2.187	2.083
1000	2.761 2.800	2.781	2.676



(pg/mL)	O.D	Average	Corrected
0	0.052 0.053	0.053	-
7.8	0.128 0.129	0.129	0.076
15.6	0.207 0.200	0.204	0.151
31.2	0.340 0.333	0.337	0.284
62.5	0.599 0.602	0.601	0.548
125	1.035 1.039	1.037	0.985
250	1.698 1.694	1.696	1.644
500	2.495 2.485	2.490	2.438

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	256.0	4.4	1.7	1	24	270.1	6.6	2.4
2	20	62.8	0.8	1.3	2	24	63.8	1.9	3.0
3	20	14.4	0.5	3.4	3	24	15.1	0.8	5.0

Recovery

The recovery of IL3 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	79	72-86
	1:4	84	74-97
Cell culture supernatants	1:2	83	73-90
	1:4	85	79-95

Sample Values

Serum - eighty serum samples from healthy volunteers were evaluated for the presence of human IL3 in this assay. seventy-nine samples measured less than the lowest standard, 15.6 pg/mL. One sample measured 444.5 pg/mL.

Cell Culture supernatants - Human peripheral blood mononuclear cells (PBMC) (1×10^6 cells/mL) were cultured in DMEM supplemented with 8% fetal bovine serum, 5 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA for 1 day. Aliquots of the cell culture supernatants were removed and assayed for levels of human IL3.

Condition	(pg/mL)
Unstimulated for 1d	-
Stimulated for 1d	127.1

Sensitivity

The minimum detectable dose of human IL3 is 0.18 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, serum and plasma samples were spiked with high concentrations of human IL3 and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. Cell culture supernatants were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma (PT 5B3)	Cell culture supernatants (PT 4B1)
1:2	Average% of Expected	99	100
	Range (%)	88-110	-
1:4	Average% of Expected	96	108
	Range (%)	87-107	101-112
1:8	Average% of Expected	90	110
	Range (%)	86-98	103-115
1:16	Average% of Expected	80	108
	Range (%)	75-84	98-117

References

1. Huixian Hong. et al. (2013) Biochem Biophys Res Commun. 440(4):545-50.
2. Y C Yang. et al. (1989) Hematol Oncol Clin North Am. 3(3):441-52.
3. B E Barton. et al. (1989) J Immunol.143(10):3211-6.