proteintech®
Antibodies | ELISA kits | Proteins

AuthentiKine™

Human LIF Sandwich ELISA Kit Datasheet

For the quantitative detection of Human LIF in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00208
Product Name	AuthentiKine™ Human LIF Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

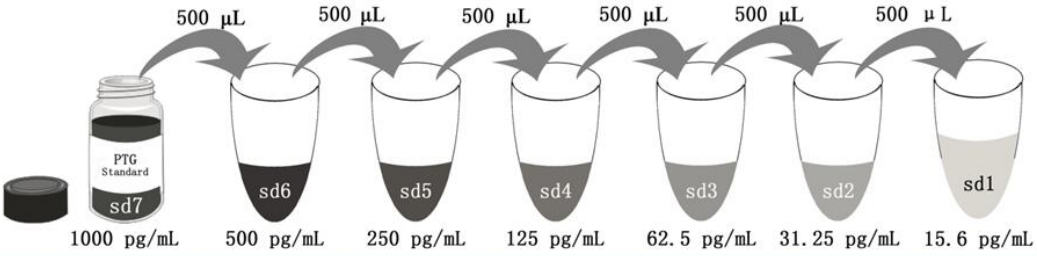
Entrez Gene	3976
SwissProt	P15018

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 5-ef - 30 mL/bottle. For serum and plasma	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants	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	

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

NB: Do not use the kit after the expiration date.
Sample Diluent PT **5-ef** is for protein standard, serum, plasma and cell culture supernatants.
Sample Diluent PT **1-ef** is for protein standard, urine and saliva.
Detection Diluent is for Detection antibody.
*Add **2 mL** Sample Diluent PT **5-ef** or PT **1-ef** 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 5-ef or PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00208 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The LIF ELISA kit is to be used to detect and quantify protein levels of endogenous LIF. The assay recognizes human LIF. An antibody specific for LIF has been pre-coated onto the microwells. The LIF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for LIF is added to detect the captured LIF 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

Leukemia inhibitory factor (LIF) is a multi-functional cytokine of the interleukin-6 (IL-6) superfamily. It utilises a receptor that consists of the LIF receptor β and gp130 and this receptor complex is also used by ciliary neurotrophic growth factor (CNTF), oncostatin M, cardiotrophin1 (CT1) and cardiotrophin-like cytokine (CLC). Functionally, LIF has been implicated in a many physiological processes including the induction of hematopoietic differentiation in normal and myeloid leukemia cells, induction of neuronal cell differentiation, regulator of mesenchymal to epithelial conversion during kidney development, and may also have a role in immune tolerance at the maternal-fetal interface.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A minimum 1:2 or 1:4 dilution is recommended for serum or plasma. A minimum 1:2 or 1:4 dilution is recommended for cell culture supernatants

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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.

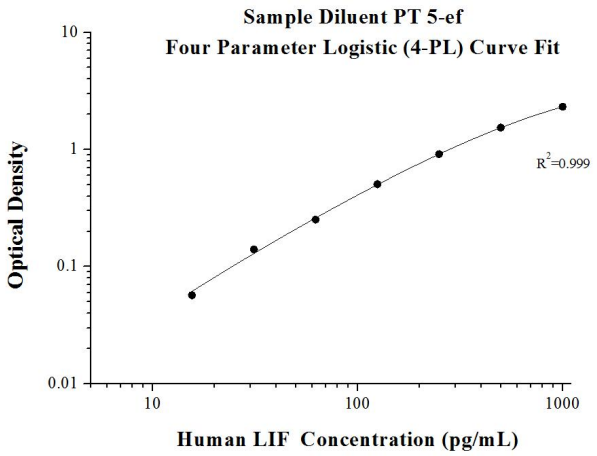
Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated 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
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.				

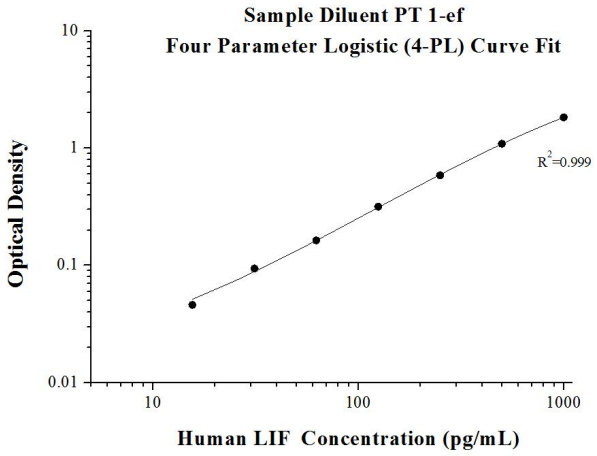
USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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	0.114 0.123	0.119	-
15.6	0.176 0.175	0.176	0.057
31.25	0.258 0.259	0.259	0.140
62.5	0.377 0.363	0.370	0.252
125	0.626 0.62	0.623	0.505
250	1.011 1.049	1.030	0.912
500	1.681 1.63	1.656	1.537
1000	2.447 2.428	2.438	2.319



(pg/mL)	O.D	Average	Corrected
0	0.062 0.06	0.061	-
15.6	0.106 0.108	0.107	0.046
31.25	0.155 0.155	0.155	0.094
62.5	0.222 0.225	0.224	0.163
125	0.374 0.38	0.377	0.316
250	0.633 0.661	0.647	0.586
500	1.147 1.15	1.149	1.088
1000	1.877 1.901	1.889	1.828

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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	171.7	7.24	4.2
2	20	332.8	14.31	4.3
3	20	711.2	38.59	5.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	160.8	4.99	3.1
2	24	329.5	11.83	3.6
3	24	696.0	29.38	4.2

Recovery

The recovery of LIF spiked to three different levels in four samples throughout the range of the assay in human samples were evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	74	70-79
	1:4	81	75-91
Human serum	1:2	79	76-83
	1:4	81	81-85
Cell culture supernatants	1:2	82	76-91
	1:4	87	81-96

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

Sample Values

Human serum samples were evaluated for human LIF in this assay.

Sample Type	Mean of Detectable (pg/mL)	%Detectable	Range (pg/mL)
Human serum (n=16)	4.6	37.5%	ND*-31.9

ND*=Non-detectable

Cell Culture supernatants - Human peripheral blood mononuclear cells (PBMC) (1 × 10^6 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. The cell culture supernatants were stimulated with 10 ug/mL of PHA. An aliquot of the culture supernatants were removed, assayed for human LIF.

Stimulated conditions	Day 1 (pg/mL)	Day 5 (pg/mL)
PHA 10 ug/mL	1259	3668
Unstimulated	10	230

Sensitivity

The minimum detectable dose of human LIF is 0.4 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 LIF 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 serum (PT 5-ef)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	85	100
	Range (%)	70-95	-
1:4	Average% of Expected	108	104
	Range (%)	88-129	98-104
1:8	Average% of Expected	120	115
	Range (%)	107-127	98-129
1:16	Average% of Expected	116	96
	Range (%)	108-121	87-102

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

References

1. D J Hilton. et al. (1991) J Cell Biochem. 46(1): 21-6.
2. Nicos A. et al. (2005) Cytokine & Growth Factor Reviews. 26(5): 533-544.
3. C J Auernhammer. et al. (2000) Endocr Rev. 21(3): 313-45.

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com