

Human IL-6 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00139

Size: 96T

Sensitivity: 1.2 pg/mL

Range: 7.8-500 pg/mL

Usage: For the quantitative detection of human IL-6 concentrations in serum, plasma and cell culture supernatant.

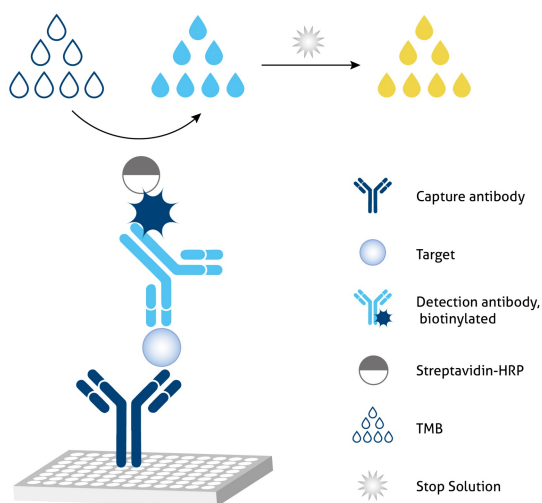
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1. Background

Interleukin-6 (IL6) is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. IL6 protein is secreted by a variety of cell types including T cells and macrophages as phosphorylated and variably glycosylated molecule. IL6 Plays an essential role in the final differentiation of B-cells into Ig-secreting cells involved in lymphocyte and monocyte differentiation. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation Acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. IL6 is also considered a myokine, a cytokine produced from muscle, and is elevated in response to muscle contraction. IL6 has been shown to interact with interleukin-6 receptor and glycoprotein 130. Additionally, IL6 is involved in hematopoiesis, bone metabolism, and cancer progression, and has been defined an essential role in directing transition from innate to acquired immunity.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and 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, biotinylated (100×) - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 1-ag - 30 mL/bottle. For human serum and plasma samples.	1 bottle	
Sample Diluent PT 4 - 30 mL/bottle. For cell culture supernatant.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

- 6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000×g. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500×g and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

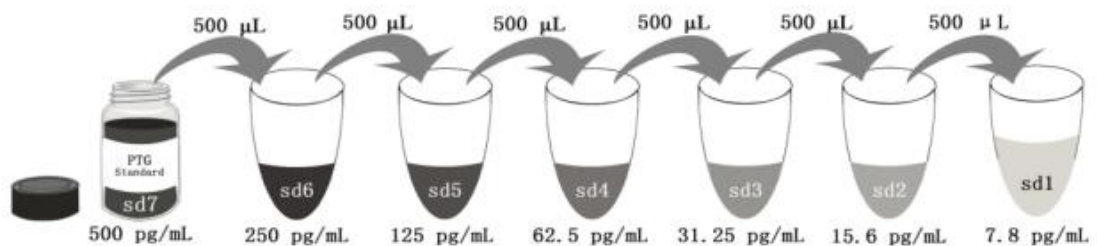
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for human serum and plasma.

7.5 Standard Serial Dilution:

For human serum and plasma samples, add 2 mL Sample Diluent PT 1-ag in protein standard; For cell culture supernatant, add 2 mL Sample Diluent PT 4 in protein standard.



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 PT4 or PT 1-ag	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, it is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

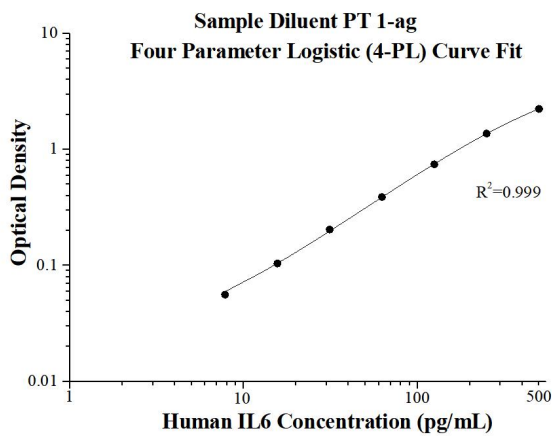
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve-fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

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.				

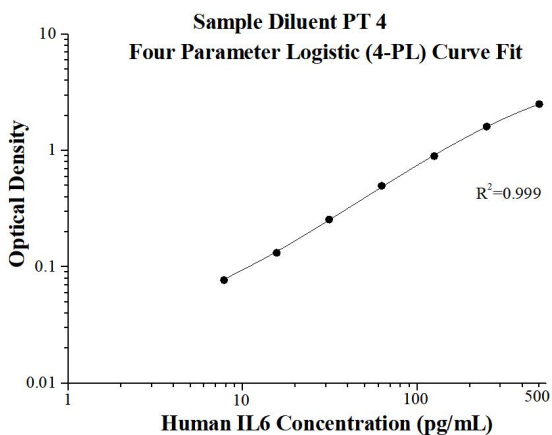
9. Validation Data

9.1 Standard curve

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	0.111 0.112	0.112	-
7.8	0.167 0.168	0.168	0.056
15.63	0.213 0.218	0.216	0.104
31.25	0.309 0.322	0.316	0.204
62.5	0.491 0.51	0.501	0.389
125	0.848 0.864	0.856	0.745
250	1.481 1.487	1.484	1.373
500	2.348 2.355	2.352	2.24



(pg/mL)	O.D	Average	Corrected
0	0.054 0.052	0.053	-
7.8	0.134 0.126	0.13	0.077
15.63	0.183 0.187	0.185	0.132
31.25	0.307 0.31	0.309	0.256
62.5	0.548 0.553	0.551	0.498
125	0.953 0.949	0.951	0.898
250	1.682 1.65	1.666	1.613
500	2.594 2.538	2.566	2.513

9.2 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	270.7	9.4	3.5	1	24	288.4	16.5	5.7
2	20	64.5	3.6	5.6	2	24	63.3	2.0	3.2
3	20	14.9	0.6	3.7	3	24	14.8	0.5	3.5

9.3 Recovery

The recovery of human IL6 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	85	81-94
	1:4	92	88-103
Cell culture supernatant	1:256	97	86-106
	1:512	98	93-102

9.4 Sample values

Thirty-one serum samples from volunteers were evaluated for human IL6 in this assay.

Sample Type	Mean of Detectable (pg/mL)	%Detectable	Range of Detectable (pg/mL)
Human serum (n=31)	22.8	71	3.1-84.4

Human peripheral blood mononuclear cells (PBMC) (1×10^6 cells/mL) were cultured in DMEM supplemented with 8% fetal bovine serum, 5µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 day and 3 days. Aliquots of the cell culture supernatant was removed and assayed for levels of human IL6.

Condition	(ng/mL)
Unstimulated for 1d	0.11
Stimulated for 1d	19
Unstimulated for 3d	0.09
Stimulated for 3d	39

9.5 Sensitivity

The minimum detectable dose of human IL6 is 1.2 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The cell culture supernatant was initially diluted 1:32)

		Human plasma (Sample Diluent PT 1-ag)	Cell culture supernatant (Sample Diluent PT 4)
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	104	100
	Range (%)	103-104	98-103
1:8	Average% of Expected	105	103
	Range (%)	104-106	100-106
1:16	Average% of Expected	104	112
	Range (%)	103-106	111-112

9.7 Calibration

The NIBSC/WHO International Standard for IL6 (89/548), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard (89/548) parallels the Proteintech standard curve. To convert sample values obtained with the human IL6 ELISA kit to approximate NIBSC 89/548 units, use the equation below.

NIBSC (89/548) approximate value (IU/mL)=0.121 × Authentikine Human IL6 value (pg/mL)

9.8 Specificity

This assay recognizes natural and recombinant human IL-6.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human: Recombinant mouse:

CNTF IL-6
LIF IL-12
OSM
IL-12
LIF R
IL-11
G-CSF
Gp130
IL-6R

10. References

1. Scheller J. et al. (1988). Behring Inst Mitt. Behring Inst Mitt. (83):40-7.
2. Heinrich PC. et al. (2003). Biochem J. 374: 1-20.
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