



AssayMax Human Interleukin-6 (IL-6) ELISA Kit

Catalog Number EI1006-1

Introduction

Interleukin-6 (IL-6) is a cytokine of approximately 26 kDa that is synthesized by T-cells, macrophages, B-cells, fibroblasts, endothelial cells, and epithelial cells. IL-6 acts in both pro-inflammatory and anti-inflammatory ways. When released systemically it stimulates the liver to produce proteins, such as C-reactive protein and fibrin, that are responsible for the acute-phase response (1). Besides the systemic acute phase reaction, IL-6 is associated with several acute and chronic inflammatory diseases, including rheumatoid arthritis, acute pancreatitis, viral and bacterial meningitis, and Alzheimer's disease (2, 3). However, IL-6 can also downregulate the inflammatory reaction by suppressing the pro-inflammatory cytokines IL-1 and TNF, and protect against lung damage (4) and septic shock (5).

Principal of the Assay

The AssayMax Human IL-6 ELISA kit is designed for detection of IL-6 in human plasma, serum or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures IL-6 in 5 hours. A murine monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. IL-6 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human IL-6, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **IL-6 Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against IL-6.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **IL-6 Standard:** Recombinant human IL-6 in a buffered protein base (1.5 ng, lyophilized).

- **Biotinylated IL-6 Antibody (100x):** A 100-fold biotinylated polyclonal antibody against IL-6 (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x):** A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8⁰C. Store reconstituted hIL-6 standard at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along with zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:2 with MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Store serum at -20⁰C or below. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent Concentrate 1:10 with reagent grade water.
- **Standard Curve:** Reconstitute the 1.5 ng of human IL-6 Standard with 1.5 ml of MIX Diluent to generate a solution of 1 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially

diluting the IL-6 standard solution twofold with equal volume of MIX Diluent to produce 0.5, 0.25, 0.125, 0.063, 0.031 and 0.016 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[IL-6] (ng/ml)
P1	Standard (1 ng/ml)	1.000
P2	1 part P1 + 1 part MIX Diluent	0.500
P3	1 part P2 + 1 part MIX Diluent	0.250
P4	1 part P3 + 1 part MIX Diluent	0.125
P5	1 part P4 + 1 part MIX Diluent	0.063
P6	1 part P5 + 1 part MIX Diluent	0.031
P7	1 part P6 + 1 part MIX Diluent	0.016
P8	MIX Diluent	0.000

- **Biotinylated IL-6 Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated IL-6 Antibody to each well and incubate for two hours.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**.

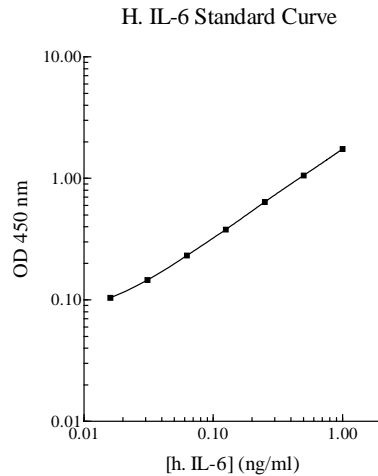
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.

- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Precision, Sensitivity and Specificity

- The minimum detectable dose of IL-6 is typically < 10 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1% and 7.0% respectively.
- This assay recognizes both natural and recombinant human IL-6.

Cross-Reactivities

Species	% Cross Reactivity
Monkey	None
Mouse	None
Rat	None
Swine	None
Beagle	None
Bovine	< 2

Recovery

Standard Added Value	0.02 – 0.5 ng/ml
Recovery %	83-115 %
Average Recovery %	99 %

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
No Dilution	102%	97%
1:2	100%	101%
1:4	101%	100%

References

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3. Feldmann, M. *et al.* (1996) *Annu Rev. Immunol.* 14:397
4. Schindler, R. *et al.* (1990) *Blood* 75:40
5. Barton, B.E. and Jackson, J.V. (1993) *Infect Immun.* 61:1496

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