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

Catalog Number EI1006-1
Lot #

## 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, tissue extracts or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures IL-6 in 3.5 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 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 (120 µl).
- **EIA 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 2-8$^0$C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8$^0$C. Store reconstituted hIL-6 standard at -20$^0$C 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 2,000x g for 10 minutes and assay. Dilute samples 1:4 with EIA Diluent. Store samples at -20$^0$C or below. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000x g for 10 minutes. Remove serum and assay. Store serum at -20$^0$C or below. Avoid repeated freeze-thaw cycles. Dilute samples 1:4 with EIA diluent before assay.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20$^0$C 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.
- **EIA Diluent Concentrate (10x)**: Dilute the EIA Diluent Concentrate 1:10 with reagent grade water.
- **Standard Curve:** Reconstitute the 1 ng of human IL-6 Standard with 1 ml of EIA 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 EIA Diluent to produce 0.5, 0.25, 0.125, 0.063, 0.031 and 0.016 ng/ml. EIA 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 EIA Diluent | 0.500 |
| P3 | 1 part P2 + 1 part EIA Diluent | 0.250 |
| P4 | 1 part P3 + 1 part EIA Diluent | 0.125 |
| P5 | 1 part P4 + 1 part EIA Diluent | 0.063 |
| P6 | 1 part P5 + 1 part EIA Diluent | 0.031 |
| P7 | 1 part P6 + 1 part EIA Diluent | 0.016 |
| P8 | EIA Diluent | 0.000 |

- **Biotinylated IL-6 Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA 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 EIA 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$^0$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 complete remove liquid at each step.
- Add 50 µl of Biotinylated IL-6 Antibody to each well and incubate for 60 minutes.
- 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**.

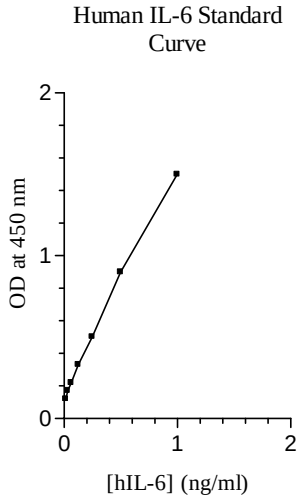
## Data Analysis

- 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 plasma or tissue value by the dilution factor of 4.

## Standard Curve

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




## Sensitivity and Specificity

- The minimum detectable dose of IL-6 is typically < 10 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 6.2% and 8.8% respectively.
- This assay recognizes both natural and recombinant human IL-6.

## References

1. Dosquet, C. *et al.* (1994) *Eur. J. Cancer* 30A:162
2. Odeh, M. (1997) *Clin. Immunol. Immunopathol*. 83:103
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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