



# Human IL-6 ELISA Kit

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**Hinweis/Note:**

Der Packungsbeileger dient nur als erste Information.  
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.  
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at [support@assaypro.com](mailto:support@assaypro.com).

Thank you for choosing Assaypro.

## Assay Summary

**Step 1.** Add 50  $\mu$ l of Standard or Sample per well.  
Incubate 2 hours.

**Step 2.** Wash, then add 50  $\mu$ l of Biotinylated Antibody per well.  
Incubate 2 hours.

**Step 3.** Wash, then add 50  $\mu$ l of SP Conjugate per well.  
Incubate 30 minutes.

**Step 4.** Wash, then add 50  $\mu$ l of Chromogen Substrate per well.  
Incubate 25 minutes.

**Step 5.** Add 50  $\mu$ l of Stop Solution per well.  
Read at 450 nm immediately.

## Symbol Key



Consult instructions for use.





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

Catalog No. EI1006-1  
Sample Insert/Reference Only

## 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, which 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 down regulate the inflammatory reaction by suppressing the pro-inflammatory cytokines IL-1 and TNF, and protect against lung damage, (4) and septic shock (5).

## Principle of the Assay

The AssayMax Human Interleukin-6 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of IL-6 in human **plasma, serum, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures IL-6 in less than 5 hours. A murine monoclonal antibody specific for human IL-6 has been pre-coated onto a 96-well microplate with removable strips. 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

- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.

- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- The Stop Solution is an acidic solution.
- This kit is for research use only.
- The kit should not be used beyond the expiration date.

## Reagents

- **Human 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 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human IL-6 Standard:** Human IL-6 in a buffered protein base (0.5 ng, lyophilized).
- **Biotinylated Human IL-6 Antibody (70x):** A 70-fold concentrated biotinylated polyclonal antibody against IL-6 (100  $\mu$ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80  $\mu$ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

## Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

## Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.

- Pipettes (1-20  $\mu$ l, 20-200  $\mu$ l, 200-1000  $\mu$ l, 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 3000 x *g* for 10 minutes and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes. Remove serum and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 0.5 ng of Human IL-6 Standard with 1 ml of MIX Diluent to generate a 0.5 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution 1:2 with MIX Diluent to produce 0.25, 0.125, 0.063, 0.031, 0.016, and 0.008 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[IL-6] (ng/ml)
P1	Standard (0.5 ng/ml)	0.500
P2	1 part P1 + 1 part MIX Diluent	0.250
P3	1 part P2 + 1 part MIX Diluent	0.125
P4	1 part P3 + 1 part MIX Diluent	0.063
P5	1 part P4 + 1 part MIX Diluent	0.031
P6	1 part P5 + 1 part MIX Diluent	0.016
P7	1 part P6 + 1 part MIX Diluent	0.008
P8	MIX Diluent	0.000

- **Biotinylated Human IL-6 Antibody (70x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 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. Any remaining solution should be frozen at -20°C.

## Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human IL-6 Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human IL-6 Antibody to each well and incubate for 2 hours.
- Wash the microplate as described 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 the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 25 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**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points



after stopping the reaction for about 10 minutes, which will reduce the readings.

## Data Analysis

- Calculate the mean value of the duplicate or 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 using four-parameter or log-log logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the dilution factor.

## Typical Data

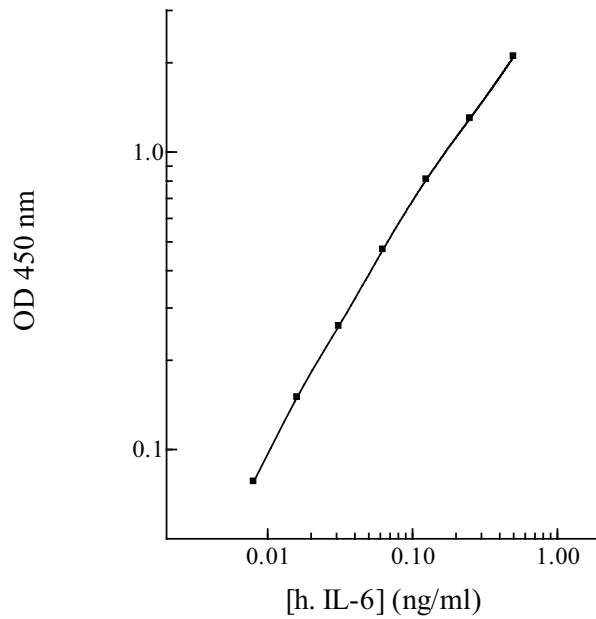
- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	0.500	1.720 1.778	1.749
P2	0.250	1.102 1.148	1.125
P3	0.125	0.675 0.642	0.658
P4	0.063	0.414 0.365	0.389
P5	0.031	0.201 0.214	0.207
P6	0.016	0.124 0.119	0.121
P7	0.008	0.075 0.069	0.072
P8	0.000	0.021 0.025	0.023
<b>Sample: Normal, Sodium Citrate Plasma (1x)</b>		0.141 0.136	0.138

## Standard Curve

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

### H. IL-6 Standard Curve



### Performance Characteristics

- The minimum detectable dose of IL-6 as calculated by 2SD from the mean of a zero standard was established to be 0.007 ng/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	2.8%	2.1%	3.0%	7.1%	7.2%	8.0%
Average CV (%)	2.6%			7.4%		

### Recovery

Standard Added Value	0.06 – 0.2 ng/ml
Recovery %	92 – 109%
<b>Average Recovery %</b>	<b>102%</b>

## Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100%

## Troubleshooting

Issue	Causes	Course of Action
<b>Low Precision</b>	Use of expired components	<ul style="list-style-type: none"> <li>• Check the expiration date listed before use.</li> <li>• Do not interchange components from different lots.</li> </ul>
	Improper wash step	<ul style="list-style-type: none"> <li>• Check that the correct wash buffer is being used.</li> <li>• Check that all wells are dry after aspiration.</li> <li>• Check that the microplate washer is dispensing properly.</li> <li>• If washing by pipette, check for proper pipetting technique.</li> </ul>
	Splashing of reagents while loading wells	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> </ul>
	Inconsistent volumes loaded into wells	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Insufficient mixing of reagent dilutions	<ul style="list-style-type: none"> <li>• Thoroughly agitate the Standard and Fluorescent Probe after reconstitution.</li> <li>• Thoroughly mix dilutions.</li> </ul>
	Improperly sealed microplate	<ul style="list-style-type: none"> <li>• Check the microplate pouch for proper sealing.</li> <li>• Check that the microplate pouch has no punctures.</li> <li>• Check that three desiccants are inside the microplate pouch prior to sealing.</li> </ul>
<b>Unexpectedly Low or High Signal Intensity</b>	Microplate was left unattended between steps	<ul style="list-style-type: none"> <li>• Each step of the procedure should be performed uninterrupted.</li> </ul>
	Omission of step	<ul style="list-style-type: none"> <li>• Consult the provided procedure for complete list of steps.</li> </ul>
	Steps performed in incorrect order	<ul style="list-style-type: none"> <li>• Consult the provided procedure for the correct order.</li> </ul>
	Insufficient amount of reagents added to wells	<ul style="list-style-type: none"> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Wash step was skipped	<ul style="list-style-type: none"> <li>• Consult the provided procedure for all wash steps.</li> </ul>
	Improper wash buffer	<ul style="list-style-type: none"> <li>• Check that the correct wash buffer is being used.</li> </ul>
	Improper reagent preparation	<ul style="list-style-type: none"> <li>• Consult reagent preparation section for the correct dilutions of all reagents.</li> </ul>
	Insufficient or prolonged incubation periods	<ul style="list-style-type: none"> <li>• Consult the provided procedure for correct incubation time.</li> </ul>

<b>Deficient Standard Curve Fit</b>	Non-optimal sample dilution	<ul style="list-style-type: none"> <li>• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay.</li> <li>• Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay.</li> <li>• User should determine the optimal dilution factor for samples.</li> </ul>
	Contamination of reagents	<ul style="list-style-type: none"> <li>• A new tip must be used for each addition of different samples or reagents during the assay procedure.</li> </ul>
	Contents of wells evaporate	<ul style="list-style-type: none"> <li>• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.</li> </ul>
	Improper pipetting	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Insufficient mixing of reagent dilutions	<ul style="list-style-type: none"> <li>• Thoroughly agitate the lyophilized components after reconstitution.</li> <li>• Thoroughly mix dilutions.</li> </ul>

## 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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