# Human IL-6 ELISA Kit

## EH2IL6

| Number | Description |
|---|---|
| **EH2IL6** | **Human Interleukin-6 (IL-6) ELISA,** sufficient reagents for 96 determinations |

| Kit Contents | EH2IL6 |
|---|---|
| Anti-human IL-6 96-well Strip Plate | 1 each |
| Recombinant Human IL-6 Standard | 2 vials |
| Standard Diluent, contains 0.1% sodium azide | 25mL |
| Biotinylated Antibody Reagent, contains 0.1% sodium azide | 8mL |
| 30X Wash Buffer | 50mL |
| Streptavidin-HRP Concentrate | 75µL |
| Streptavidin-HRP Dilution Buffer | 14mL |
| TMB Substrate | 13mL |
| Stop Solution, contains 0.16M sulfuric acid | 13mL |
| Adhesive plate covers | 6 each |

For research use only. Not for use in diagnostic procedures.

**Storage:** Upon receipt store the kit at 2-8ºC.

## Table of Contents

## Introduction

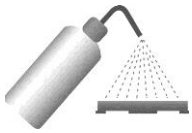
The Thermo Scientific™ Human Interleukin-6 (IL-6) ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human IL-6 in serum, plasma, urine and culture supernatants.

## Procedure Summary

**1.** Add 50µL of Biotinylated Antibody Reagent to each well.

**2.** Add 50µL of Standards or samples to each well in duplicate.

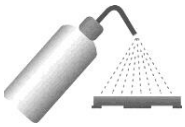

**3.** Cover plate and incubate at room temperature (20-25°C) for 2 hours.



**4.** Wash plate THREE times.

**5.** Add 100µL of prepared Streptavidin-HRP Solution to each well.


**6.** Cover plate and incubate at room temperature for 30 minutes.



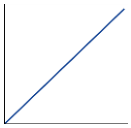
**7.** Wash plate THREE times.

**8.** Add 100µL of TMB Substrate to each well.


**9.** Develop plate in the dark at room temperature for 30 minutes.


**10.** Stop reaction by adding 100µL of Stop Solution to each well.

**11.** Measure absorbance on a plate reader at 450nm or 450 minus 550nm.



**12.** Calculate the results using graph paper or curve-fitting statistical software.

## Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-l000µL
- Plastic pipettes to deliver 5-15mL
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

## Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use in the assay.**
- Review these instructions carefully and verify all components against the contents list (page 1) before beginning.
- Do not use a water bath to thaw samples. Thaw at room temperature.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute the standard and samples. Do NOT use RPMI without serum supplement.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Avoid microbial contamination of reagents.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Discard unused ELISA components after assay completion. Do not mix reagents from different kit lots.
- Do not use glass pipettes to measure TMB Substrate Solution. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some kit components contain sodium azide. Please dispose of reagents according to local regulations.

## Sample Preparation

- Serum; EDTA, heparin and sodium citrate plasma; urine; and culture supernatants may be tested in this ELISA.
- 50µL per well of serum, plasma, urine or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeated freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use a heated water bath to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.
- If the human IL-6 concentration possibly exceeds the highest point of the standard curve (i.e., 400pg/mL), prepare one or more 10-fold dilutions of the test sample. When testing culture supernatants, prepare the serial dilutions using your culture medium. When testing serum, plasma or urine prepare the serial dilutions using the Standard Diluent provided. For example, a 10-fold dilution is prepared by adding 0.05mL (50µL) of test sample to 0.45mL (450µL) of appropriate diluent. Mix thoroughly between dilutions before assaying.

## Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout these instructions.

### Wash Buffer

1. Label a clean glass or plastic two-liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. **(PP)** When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

   **Note:** Wash buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

### Standards

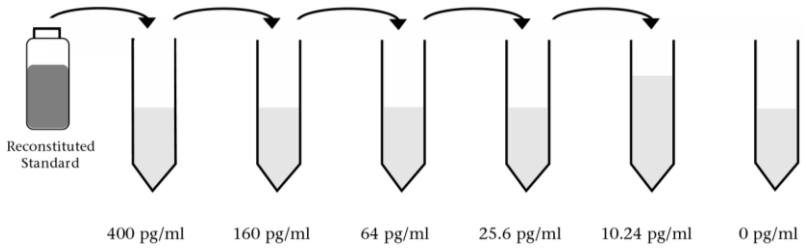
- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

1. When testing **culture supernatant samples,** reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the sample culture medium to prepare Standard Curve dilutions.

   When testing **serum, plasma or urine samples,** reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the Standard Diluent provided to prepare standard curve serial dilutions.

   When testing **serum, plasma or urine and cell culture supernatant samples on the same plate,** validate the media to establish if the same standard curve can be used for the different sample types. Prepare a standard curve (including a zero/blank) using culture medium to reconstitute and dilute the standard. Use medium containing serum or other protein to maximize stability of the human IL-6. Perform this curve in parallel with a standard curve prepared with Standard Diluent. If the OD values of the two curves are within 10% of the mean for both curves, the assay can be performed with Standard Diluent, whether you are testing culture supernatant, urine, plasma or serum samples.
2. Label six tubes, one for each standard curve point: 400, 160, 64, 25.6, 10.24 and 0pg/mL, then prepare 1:2.5 serial dilutions for the standard curve as follows:
3. Pipette 240µL of appropriate diluent into each tube.
4. Pipette 160µL of the reconstituted standard into the first tube (i.e., 400pg/mL) and mix.
5. Pipette 160µL of this dilution into the second tube (i.e., 160pg/mL) and mix.
6. Repeat the serial dilutions (using 160 µL) three more times to complete the standard curve points. These concentrations, 400pg/mL, 160pg/mL, 64pg/mL, 25.6pg/mL, 10.24pg/mL and 0pg/mL, are the standard curve.

**Serial dilutions using 160µL**



## Assay Procedure

### A. Biotinylated Antibody Reagent and Sample Incubation

- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Place remaining unused strips in the provided foil pouch with desiccant. Make sure foil pouch is sealed tightly. After completing assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.
- Use the Data Template provided to record the locations of the zero standard (blank or negative control), IL-6 standards and test samples. Perform five standard points and one blank in duplicate with each series of unknown samples.
- If using a multichannel pipettor, use a new reagent reservoir to add the Biotinylated Antibody Reagent. Remove from the vial only the amount required for the number of strips being used. Take care not to touch the samples in wells with the pipette tip when adding the Biotinylated Antibody Reagent.

1. Add 50µL of Biotinylated Antibody Reagent to each well.
2. Add 50µL of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

   **Note:** If the human IL-6 concentration in any test sample possibly exceeds the highest point on the standard curve, 400pg/mL, see Sample Preparation Section.
3. Add 50µL of Standard Diluent to all wells that do not contain standards or samples.
4. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate for two (2) hours at room temperature, 20-25°C.
5. Carefully remove adhesive plate cover. Wash plate THREE times with Wash Buffer as described in the Plate Washing section below.

### B. Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate onto paper towels or other absorbent material.

   **Note:** For automated washing, aspirate all wells and wash THREE times with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

### C. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use.** Do not prepare more solution than is required.
- Do not store prepared Streptavidin-HRP Solution.
- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
- If using a multichannel pipettor, **use a new reagent reservoir and new pipette tips** when adding the prepared Streptavidin-HRP Solution.

1. Briefly centrifuge Streptavidin-HRP Concentrate to force entire vial contents to the bottom.
2. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5µL of Streptavidin-HRP Concentrate with 1 ml of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

   For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.
3. Add 100µL of prepared Streptavidin-HRP Solution to each well.

4. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate the plate for 30 minutes at room temperature, 20-25°C.
5. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing Section.

**D. Substrate Incubation and Stop Step**

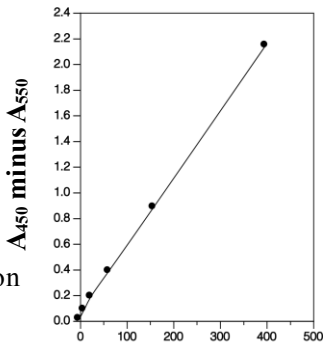
- Use new disposable reagent reservoirs when adding TMB Substrate Solution and Stop Solution.
- Dispense from bottle ONLY amount required, 100µL per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate Solution.
- **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate Solution.

1. Pipette 100µL of TMB Substrate into each well. Allow color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
2. After 30 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

**E. Absorbance Measurement**

**Evaluate the plate within 30 minutes of stopping the reaction.** Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If 550nm is not available, measure at 450nm only. Omitting the 550nm measurement will result in higher absorbance values.

**Standard Curve Example**




**F. Results Calculation**

- Use the standard curve to determine human IL-6 amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding sample concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. The human IL-6 amount in each sample is determined by interpolating from the absorbance value (Y axis) to human IL-6 concentration (X axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/ml of human IL-6 in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

## Performance Characteristics

**Sensitivity:** < 1pg/mL
The sensitivity or Lower Limit of Detection (LLD)[1] was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

**Assay Range:** 10.24-400pg/mL (Standard curve points are 400, 160, 64, 25.6, 10.24, and 0pg/mL.)

**Reproducibility:** Intra-assay CV: < 10% Inter-assay CV: < 10%

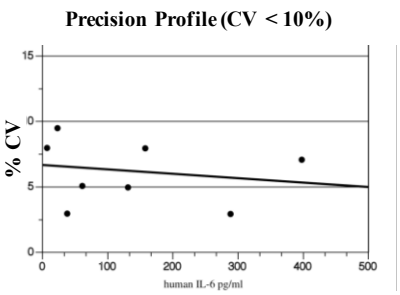
**Specificity:** This ELISA is specific for the measurement of natural and recombinant human IL-6. It does not cross-react with human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12, TNFα, IFNγ, IFNα, GM-CSF, or mouse IL-6.

**Calibration:** The standards in this ELISA have been re-calibrated to the NIBSC recombinant IL-6 reference standard lot 89/548. One (1) pg of Standard = 3 NIBSC pg = 0.3 NIBSC units.

**Expected Values:**

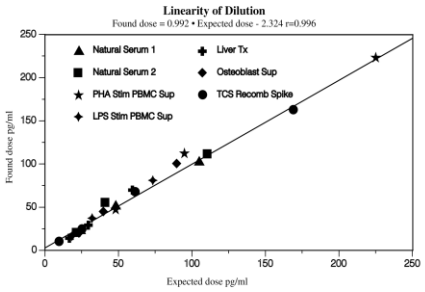
| Sample type & Number | Mean | Range |
|---|---|---|
| serum (n=14) | 43pg/mL | 0-149pg/mL |
| plasma (n=14) | 0.7pg/mL | 0-5pg/mL |
| urine (n=5) | 0.3pg/mL | 0-0.6pg/mL |

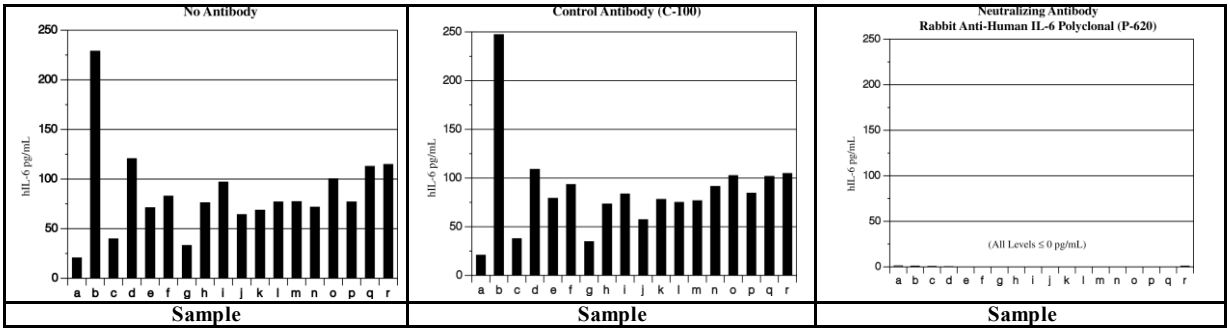
**Precision:** The intra-assay coefficient of variation is plotted against IL-6 concentration (pg/mL). The points represent samples evaluated in replicates of four in four different kit lots.




**Linearity of Dilution:** Linearity of dilution was determined by serially diluting seven different positive samples. The dilutions were evaluated in the ELISA and the "found" doses are plotted against the "expected" doses.

**Neutralization of Human IL-6 "Positive Samples":**
The specificity of this ELISA was confirmed by performing neutralization experiments. Eighteen human IL-6 "positive samples" were incubated with either no antibody, a neutralizing rabbit anti-human IL-6 polyclonal (P-620) or a control IgG (C-100). These samples were then evaluated in the ELISA.



## Cited Reference

1. *Immunoassay: A Practical Guide,* Chan and Perlstein, Eds., 1987, Academic Press: New York, p71.

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**Product label explanation of symbols and warnings**

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REF | Catalog Number | LOT | Batch code |  | Temperature limitation |  | Use by |  | Manufacturer |  | Consult instructions for use |  | Caution, consult accompanying documents |

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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