INSTRUCTIONS

# Human IL-12 (p70) ELISA Kit

## EHIL12 EHIL122 EHIL125

1361.4

| Number | Description |
|---|---|
| **EHIL12** | **Human Interleukin-12 (IL-12) p70 ELISA Kit,** sufficient reagents for 96 determinations |
| **EHIL122** | **Human Interleukin-12 (IL-12) p70 ELISA Kit,** sufficient reagents for 2 × 96 determinations |
| **EHIL125** | **Human Interleukin-12 (IL-12) p70 ELISA Kit,** sufficient reagents for 5 × 96 determinations |

| Kit Contents | EHIL12 | EHIL122 | EHIL125 |
|---|---|---|---|
| Anti-human IL-12 Precoated 96-well Strip Plate | 1 each | 2 each | 5 each |
| Lyophilized Recombinant Human IL-12 Standards | 2 vials | 4 vials | 5 vials |
| Standard Diluent | 14mL | 2 × 14mL | 75mL |
| Biotinylated Antibody Reagent | 8mL | 2 × 8mL | 35mL |
| 30X Wash Buffer | 50mL | 2 × 50mL | 200mL |
| Streptavidin-HRP Concentrate | 75µL | 2 × 75µL | 250µL |
| Streptavidin-HRP Dilution Buffer | 14mL | 2 × 14mL | 70mL |
| TMB Substrate | 13mL | 2 × 13mL | 5 × 13mL |
| Stop Solution, contains 0.16M sulfuric acid | 13mL | 2 × 13mL | 55mL |
| Adhesive plate covers | 6 each | 12 each | 30 each |

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

**Storage:** For maximum stability, store in a non-defrosting -20ºC freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8ºC and refer to the expiration date for refrigerated storage. Once thawed, store at 4ºC until the expiration date for refrigerated storage. Kit is shipped on dry ice.

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## Introduction

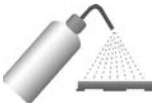
The Thermo Scientific Human Interleukin-12 (IL-12) ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of biologically active human IL-12 (p70) in serum, plasma, urine and culture supernatants.

## Procedure Summary

**1.** Add 50µL of Biotinylated Antibody Reagent to wells.

**2.** Add 50µL of standards or samples to wells in duplicate.


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



**4.** Wash plate THREE times.

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


**6.** Cover plate. 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.** Add 100µL of Stop Solution to each well.

**11.** Measure absorbance at 450nm minus 550nm.

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

## Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and plastic pipettes to deliver 5-15mL
- A glass or plastic 2L 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
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- Disposable reagent reservoirs
- 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.

## Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use.**
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning assay.
- Do not use a heated water bath to thaw samples. Thaw samples at room temperature.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute standards and samples. Do NOT use RPMI without serum supplement.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use new 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.
- Do not mix reagents from different kit lots. Discard unused kit components.

- Do not use glass pipettes to measure TMB Substrate. 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.

### Additional Precautions for the 5-plate Kit

- Dispense and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.
- Use only one bottle of the TMB Substrate per 96-well plate. Do not combine leftover substrate with that reserved for other plates.
- Use only one vial of standard per 96-well plate.

## Sample Preparation

### Sample Handling

- Serum; EDTA, heparin and sodium citrate plasma; urine; or culture supernatants may be tested in this ELISA.
- 50µL per well of serum, plasma, urine or culture supernatant are required. Mix samples by gently inverting tubes.
- 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.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use heated water baths to thaw or warm samples.
- If samples are clotted, grossly hemolyzed, lipemic or contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.
- Samples and standards must be assayed in duplicate each time the ELISA is performed.

### Sample Dilution

- If the human IL-12 concentration possibly exceeds the highest point of the standard curve (i.e., 600pg/mL), prepare one or more five-fold dilutions of the test sample. When testing **culture supernatants,** prepare serial dilutions using culture medium. When testing **serum, plasma or urine,** prepare serial dilutions using the Standard Diluent provided. A five-fold dilution is prepared by adding 50µL of sample to 200µL of appropriate diluent. Mix thoroughly between dilutions.

## Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout this instruction booklet.

**Note:** When using the 5-plate kit, only one standard per plate is supplied. Therefore, partial plates cannot be used.

### Wash Buffer

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

1. Label a clean glass or plastic two-liter container “Wash Buffer.” The 30X Wash Buffer may have a cloudy appearance.
2. If using the 5-plate kit, add 30mL Wash Buffer to 870mL water for each plate used, otherwise, add the entire contents of the 30X Wash Buffer (50mL) bottle to the 2L container and dilute to a final volume of 1.5L with ultrapure water. Mix thoroughly.

   **(PP)** When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

**Standards**

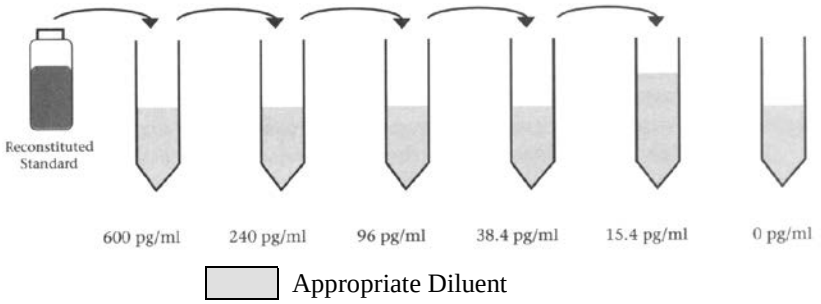
- **(PP)** Reconstitute and use one vial of the lyophilized standard per partial plate.
- Prepare standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

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 sample culture medium to prepare standard curve serial 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, urine or 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 standard. Use a medium containing serum or other protein to maximize stability of the IL-12. Perform this curve in parallel with a standard curve reconstituted in ultrapure water and diluted in the Standard Diluent provided. If OD values are within 10% of the mean for both curves, then the assay may be performed with Standard Diluent, whether testing culture supernatant, urine, plasma, or serum samples.
2. Label six tubes, one for each standard curve point: 600, 240, 96, 38.4, 15.4, 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., 600pg/mL) and mix.
5. Pipette 160µL of this dilution into the second tube (i.e., 240pg/mL) and mix.
6. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points.

**Serial dilutions using 160 µL**




## Assay Procedure

**A. Biotinylated Antibody Reagent and Sample Incubation**

- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Tightly seal remaining strips in the provided foil pouch with desiccant and store at 2-8°C. 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 locations of the zero standard (blank or negative control), human IL-12 standards and 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 IL-12 concentration in any sample possibly exceeds the highest point on the standard curve, 600pg/mL, see Sample Preparation – Sample Dilution 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 sealed tightly by running your thumb over edges and down each strip. Incubate for three (3) hours at room temperature, 20-25°C.
5. Carefully remove adhesive plate cover. Wash plate as described in the Plate Washing Section below.

### B. Plate Washing

1. Gently squeeze the long sides of the 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 Streptavidin-HRP Solution than required.
- Do not store prepared Streptavidin-HRP Solution.
- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
- **Note:** If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the prepared Streptavidin-HRP Solution.

1. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom of the vial.

   **(PP)** Use only the amount of Streptavidin-HRP Solution required for the number of strips being used. For each strip, mix 2.5µL of Streptavidin-HRP Concentrate with 1mL 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.
2. Add 100µL of prepared Streptavidin-HRP Solution to each well.
3. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
4. Carefully remove the adhesive cover, discard plate contents and wash plate 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 Solution into each well.
2. Allow color 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.
3. After 30 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

### E. Absorbance Measurement

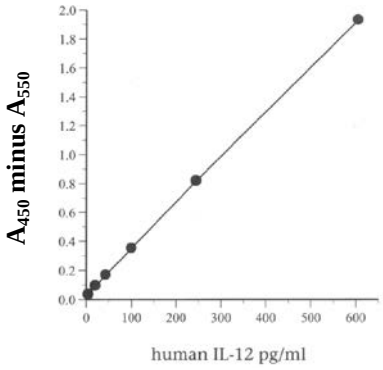
**Note:** Evaluate plate within 30 minutes of stopping the reaction.

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

### F. Calculation of Results

- Use the standard curve to determine human IL-12 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 human IL-12 concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine human IL-12 amount in each sample by interpolating from the absorbance value (Y axis) to human IL-12 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 determine pg/ml of IL-12 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%.

**Standard Curve Example**



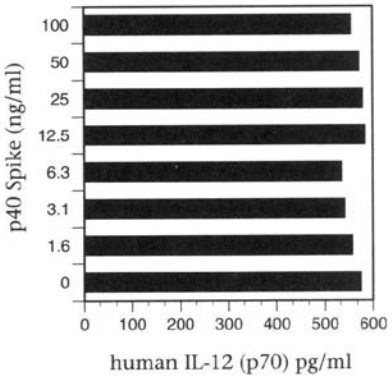

## Performance Characteristics

**Sensitivity:** < 3 pg/ml
The sensitivity or Lower Limit of Detection (LLD)[1] is 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:** 15.4-600 pg/ml
Suggested standard curve points are 600, 240, 96, 38.4, 15.4, and 0 pg/ml.

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

**p40 Does Not Interfere with the Measurement of Human IL-12 (p70)**




*Various concentrations of p40 were spiked into samples containing 570 pg/ml p70 and tested in the assay. Human p70 is plotted against p40 concentrations.*

**Specificity:** This ELISA is specific for the measurement of biologically active natural and recombinant human IL-12. Recombinant human p40 does not cross-react or interfere with this assay. This ELISA does not cross react with mouse IL-12, mouse p40 homodimer or human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-13, IL-15, TNFα, IFNγ, GM-CSF, GROα or GROβ.
**Calibration:** The standards in this ELISA are calibrated to the NIBSC recombinant IL-12 standard 95/544. One (1) pg of internal standard = 1.66 pg of NIBSC standard = 0.0166 NIBSC units.

**Precision:** The intra-assay coefficient of variation is plotted against IL-12 concentration (pg/ml). The points represent samples evaluated in replicates of four in four different kit lots.

**Expected Values:** Serum, EDTA, heparin and sodium citrate plasma, and urine samples collected from normal human donors are evaluated in this assay. The levels of human IL-12 obtained in each sample type are reported in Table 1.

**Table 1. Human IL-12 levels.**

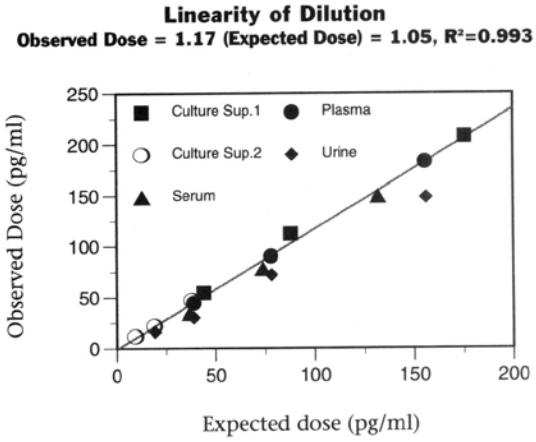
| Sample type | Average | Range |
|---|---|---|
| Serum samples (n=18) | 1.20pg/mL | 0-7.9pg/mL |
| Plasma samples (n=26) | 1.37pg/mL | 0-23.2pg/mL |
| Urine samples (n=14) | 0.36pg/mL | 0-1.4pg/mL |

**Recovery:** Cytokine recovery is determined by spiking various levels of recombinant human IL-12 into normal human serum, plasma, and urine samples and a Standard Diluent control buffer. Mean recoveries are reported in Table 2.

**Table 2. Mean recoveries.**

| Spike Level | 95pg/mL | 200pg/mL | 380pg/mL |
|---|---|---|---|
| Serum (n=8) | 93% | 101% | 98% |
| EDTA Plasma (n=8) | 82% | 93% | 96% |
| **Spike Level** | **50pg/mL** | **400pg/mL** | |
| Urine (n=8) | 63% | 67% | |

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




## Cited Reference


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

## Data Templates

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
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| H | | | | | | | | | | | | |