INSTRUCTIONS

# Total Human IL-12 ELISA Kit

## EH2IL12T EH2IL12T2 EH2IL12T5

1357.4

| Number | Description |
|---|---|
| **EH2IL12T** | **Total Human Interleukin-12 (IL-12, p40 & p70) ELISA Kit,** sufficient reagents for 96 determinations |
| **EH2IL12T2** | **Total Human Interleukin-12 (IL-12, p40 & p70) ELISA Kit,** sufficient reagents for 2 x 96 determinations |
| **EH2IL12T5** | **Total Human Interleukin-12 (IL-12, p40 & p70) ELISA Kit,** sufficient reagents for 5 x 96 determinations |

| Kit Contents | EH2IL12T | EH2IL12T2 | EH2IL12T5 |
|---|---|---|---|
| Anti-human IL-12 Total Precoated 96-well Strip Plate | 1 each | 2 each | 5 each |
| Lyophilized Natural Human IL-12 Standard | 2 vials | 4 vials | 10 vials |
| Standard Diluent | 12mL | 2 x 12mL | 5 x 12mL |
| FITC-Conjugate Reagent | 12mL | 2 x 12mL | 5 x 12mL |
| 30X Wash Buffer | 50mL | 2 x 50mL | 5 x 50mL |
| HRP-Conjugate Reagent | 12mL | 2 x 12mL | 5 x 12mL |
| TMB Substrate | 13mL | 2 x 13mL | 5 x 13mL |
| Stop Solution, contains 0.16M sulfuric acid | 13mL | 2 x 13mL | 5 x 13mL |
| Adhesive plate covers | 4 each | 8 each | 20 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™ Total Human IL-12 ELISA Kit measures human IL-12 (both p70 and p40 forms) in serum, plasma and culture supernatants.

## Procedure Summary

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

**2.** Add 100µL of FITC-Conjugate Reagent to each well.


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

**4.** Wash plate THREE times.

**5.** Add 100µL HRP-Conjugate Reagent to each well.


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

**7.** Wash plate THREE times.

**8.** Add 100µL Premixed TMB Substrate Solution 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 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
- Disposable reagent reservoirs, 4 each
- 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 ELISA.**

- Review all instructions carefully and verify all components against the Kit 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 dilutions 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.
- To avoid cross-contamination, always use a new disposable reagent reservoir and new disposable pipette tips for each transfer. Also 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 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. 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 assay to avoid contact with samples and reagents. Please follow proper disposal procedures.

## Additional Precaution for the 2-plate and 5-plate Kits

- Dispense, pool, 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.

## Sample Preparation

### Sample Handling

- Serum, EDTA and sodium citrate plasma, and culture supernatants may be tested in this ELISA. Use 50µL per well of serum, plasma or culture supernatant.
- Lower cytokine recovery is observed when using heparin anticoagulated plasma samples. For optimal results, **use EDTA or sodium citrate** anticoagulated plasma.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeat freeze-thaw cycles when storing samples.
- Samples and standards should be assayed in duplicate each time the ELISA is performed.
- 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 heated water baths 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.

### Sample Dilution

- If the human IL-12 concentration possibly exceeds the highest point of the standard curve (i.e., 1000pg/mL), prepare one or more 10-fold dilutions of the test sample. When testing **culture supernatants,** prepare the serial dilutions using the culture medium. When testing **serum or plasma,** prepare the serial dilutions using the Standard Diluent provided. For example, a 10-fold dilution is prepared by adding 50µL of test sample to 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. Add the entire contents of the 30X Wash Buffer (50mL) bottle to the two-liter 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.

   **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**

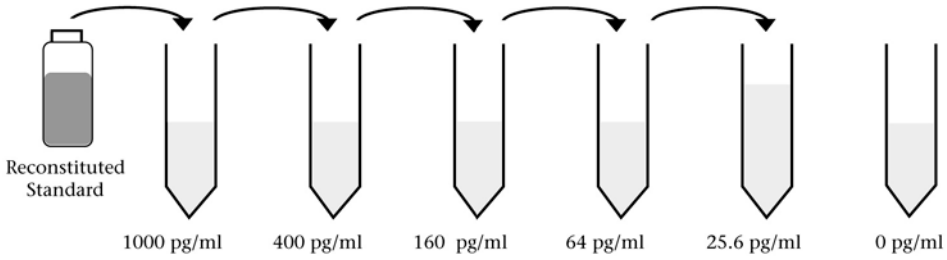
- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

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 the vial. Use the sample culture medium to prepare standard curve dilutions.

   When testing **serum or plasma 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 or plasma 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-12. Perform this curve in parallel with a standard curve prepared with the Standard Diluent provided. If the OD values of the two curves are within 10% of the mean for both curves, then the assay can be performed with Standard Diluent, whether you are testing culture supernatant, plasma, or serum samples.
2. Label six tubes, one for each standard curve point: 1000pg/mL, 400pg/mL, 160pg/mL, 64pg/mL, 25.6pg/mL, 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., 1000pg/mL) and mix.
5. Pipette 160µL of this dilution into the second tube (i.e., 400pg/mL) and mix.
6. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points.

**Serial Dilutions using 160µL**




## Assay Procedure

### A. Sample and FITC-Conjugate Reagent Incubation

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Tightly seal remaining unused 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 the 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 FITC-Conjugate 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 FITC-Conjugate Reagent.

1. Add 50µL of reconstituted standards or test samples in duplicate to each well.

   **Note:** If the human IL-12 concentration in any test sample possibly exceeds the highest point on the standard curve, 1000pg/mL, see Sample Preparation–Sample Dilution Section.
2. Add 100µL of FITC-Conjugate Reagent to each well being used. Mix well by gently tapping the plate several times.

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 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.

   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. HRP-Conjugate Reagent Incubation

- **Note:** If using a multichannel pipettor**, use new reagent reservoir and pipette tips** when adding the HRP-Conjugate Reagent.
- **(PP)** Remove from the vial only the amount of HRP-Conjugate Reagent required for the number of strips being used.

1. Add 100µL of HRP-Conjugate Reagent to each well.
2. 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.
3. 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 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.
- **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate.

1. Pipette 100µL of TMB Substrate into each well.
2. 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.
3. After 30 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

### E. Absorbance Measurement

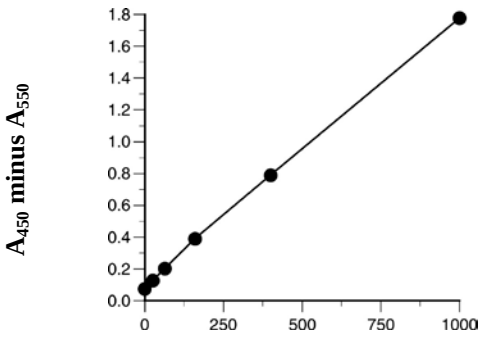
**Note: Evaluate the plate within 30 minutes of stopping the reaction.**

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

### F. Calculation of Results

- The standard curve is used 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 the 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 calculate pg/mL of human 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:** < 5pg/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:** 25.6-1000pg/mL
Suggested standard curve points are 1000, 400, 160, 64, 25.6, and 0pg/mL.

**Reproducibility:**
Intra-Assay CV: <10%
Intra-Assay CV: <10%

**Specificity:** This ELISA is specific for the measurement of natural and recombinant human IL-12. It does not cross-react with human GM-CSF, IFNα, IFNγ, TNFα, TNFβ, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-13, IL-15, mouse IL-12 (p70) or mouse IL-12 (p40).

**Calibration:** The standards in this ELISA are calibrated to a natural human IL-12 reference standard. The NIBSC recombinant IL-12 standard lot # 95/44 was tested in this assay. One (1) pg of internal standard = 1.27pg of NIBSC standard = 0.0127 NIBSC units. At the time this instruction booklet was printed no NIBSC reference was available for Total Human IL-12.

**Expected Values:** Serum and plasma samples were collected from healthy human donors and evaluated in this assay. The levels of human IL-12 found in each sample type are reported below:

| Sample Type | Average | Range |
|---|---|---|
| Serum samples (n=9) | 81pg/mL | 39-170pg/mL |
| Plasma samples (n=9) | 97pg/mL | 31-226pg/mL |

**Recovery:** Cytokine recovery was determined by spiking various levels of recombinant human IL-12 into normal human serum and plasma samples and a Standard Diluent control buffer. Mean recoveries were as follows:

| Spike Level | 150pg/mL | 500pg/mL |
|---|---|---|
| Serum (n=6) | 79% | 75% |
| Plasma (n=6) | 71% | 68% |

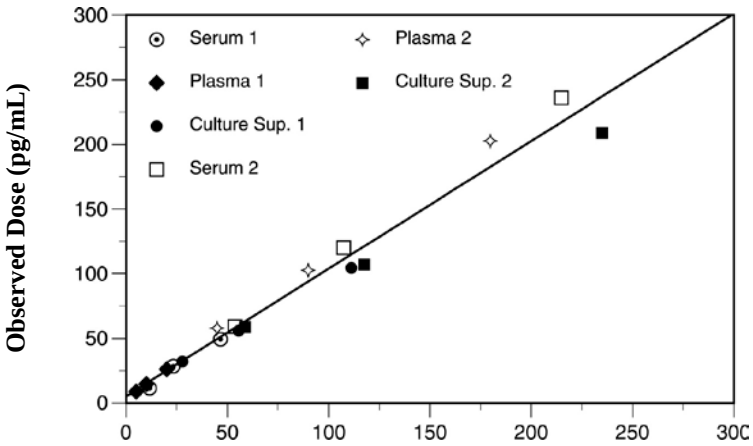
Recovery in various clinical sample matrices was determined by spiking 250pg/mL of human IL-12 into matched serum and plasma samples prepared from four healthy individual human donors and a Standard Diluent control buffer. Mean recoveries were as follows:

| Sample Type | Mean % Recovery |
|---|---|
| Serum | 84% |
| EDTA Plasma | 81% |
| Heparin Plasma | 58% |
| Sodium Citrate Plasma | 100% |

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

**Linearity of Dilution**

Observed Dose = 0.99 (Expected Dose) + 5.15, $R^2$=0.97




**Expected Dose (pg/mL)**

## 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 | | | | | | | | | | | | |
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