

# Human IL-8 ELISA Kit

**EH2IL8 EH2IL82 EH2IL85**

1349.4

Number	Description
EH2IL8	<b>Human Interleukin-8 (IL-8) ELISA</b> , sufficient reagents for 96 determinations
EH2IL82	<b>Human IL-8 ELISA</b> , sufficient reagents for 2 × 96 determinations
EH2IL85	<b>Human IL-8 ELISA</b> , sufficient reagents for 5 × 96 determinations

Kit Contents	EH2IL8	EH2IL82	EH2IL85
Anti-Human IL-8 Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Human IL-8 Standard	2 vials	4 vials	5 vials
Standard Diluent	25mL	2 × 25mL	110mL
30X Wash Buffer	50mL	2 × 50mL	200mL
Biotinylated Antibody Reagent	8mL	2 × 8mL	35mL
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 -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.


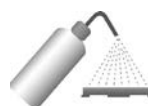










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## Introduction

The Thermo Scientific Human IL-8 ELISA Kit measures human IL-8 in serum, plasma, urine and culture supernatants.

## Procedure Summary

- |  |   |   |   |
|--|---|---|---|
| <br><b>1.</b> Add 50µL of Standards or samples to each well in duplicate. Cover plate. Incubate at room temperature (20-25°C) for 1 hour. | <br><b>2.</b> Wash plate THREE times.  | <br><b>3.</b> Add 50µL of Biotinylated Antibody Reagent to each well.       | <br><b>4.</b> Cover plate and incubate at room temperature for 1 hour.                     |
| <br><b>5.</b> Wash plate THREE times.   | <br><b>6.</b> Add 100µL of prepared Streptavidin-HRP Solution to each well.  | <br><b>7.</b> Cover and incubate plate at room temperature for 30 minutes.  | <br><b>8.</b> Wash plate THREE times.  |
| <br><b>9.</b> Add 100µL of TMB Substrate to each well.  | <br><b>10.</b> Develop plate in the dark at room temperature for 30 minutes. | <br><b>11.</b> Stop reaction by adding 100µL of Stop Solution to each well. | <br><b>12.</b> Measure absorbance on a plate reader at 450 minus 550nm. Calculate results. |

## Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and 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 all instructions carefully and verify components against the Kit contents list (page 1) before beginning the 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 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.

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- 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.

## Additional Precautions for the 5-plate Kit

- Dispense 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 Solution per 96-well plate. Do not combine leftover substrate with that reserved for other plates.
- Equilibrate to room temperature only the reagent volumes required for the number of plates being used.
- Use only one vial of Standard per 96-well plate.

## Sample Preparation

### Sample Handling

- 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 repeat 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 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-8 concentration possibly exceeds the highest point of the standard curve (i.e., 1000pg/mL), prepare one or more 20-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 20-fold dilution is prepared by adding 20µL of test sample to 380µL of appropriate diluent. Mix thoroughly between dilutions before assaying.

**Note:** When assaying culture supernatants, Pierce Endogen scientists observed nanogram and microgram quantities of human IL-8. Multiple dilutions of culture supernatant samples may be required.

## Reagent Preparation

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

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

### Wash Buffer

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.

**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.

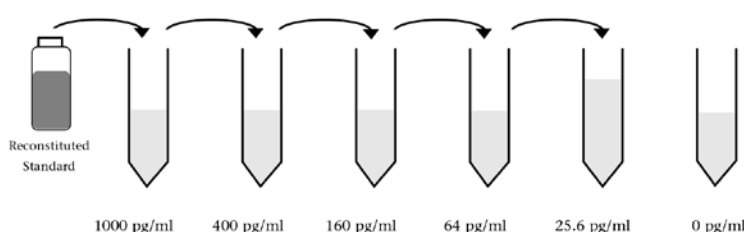
1. Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
2. When testing culture supernatant samples, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 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 both 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-8. 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, then the assay can be performed with Standard Diluent, whether you are testing culture supernatant, urine, plasma or serum samples.

3. Label six tubes, one for each standard curve point: 1000pg/mL, 400pg/mL, 160pg/mL, 64pg/mL, 25.6pg/mL and 0pg/mL. Prepare 1:2.5 serial dilutions for the standard curve as follows:
4. Pipette 240µL of appropriate diluent into each tube.
5. Pipette 160µL of the reconstituted standard into the first tube (i.e., 1000pg/mL) and mix.
6. Pipette 160µL of this dilution into the second tube (i.e., 400pg/mL) and mix.
7. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points.

#### Serial Dilutions using 160µL



## Assay Procedure

### A. Sample Incubation

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Tightly seal the remaining unused strips in the foil pouch with the desiccant provided and store at 2-8°C. After completing the assay, retain the plate frame for the second partial plate. When using the second partial plate, place the reserved strips securely in the plate frame.
  - Use the Data Template provided to record locations of the zero standard (blank or negative control), IL-8 standards and test samples. Perform five standard points and one blank in duplicate with each series of unknown samples.
1. Add 50µL reconstituted standard or sample to each well in duplicate. Mix well by gently tapping the plate several times.  
**Note:** If the IL-8 concentration in any test sample is expected to exceed the highest point on the standard curve, 1000pg/mL, refer to the Sample Dilution Section.
  2. Add 50µL of Standard Diluent to all wells that do not contain standards or samples.
  3. Carefully cover the plate with an adhesive plate cover. Ensure that all edges and strips are sealed tightly by running your thumb over the edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.
  4. Carefully remove the adhesive plate cover. Wash the plate **THREE** times with Wash Buffer using the procedure described in the Plate Washing Section (section B).

### 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. Biotinylated Antibody Incubation

1. If using a multichannel pipettor, use a new reagent reservoir to add the Biotinylated Antibody Reagent.
2. **(PP)** Remove from the vial only the amount required for the number of strips being used.
3. Add 50 µl of the Biotinylated Antibody Reagent to each well.
4. Carefully cover the plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over the edges and down each strip. Incubate for one (1) hour at room temperature, 20–25°C.
5. Carefully remove the adhesive plate cover. Wash the plate **THREE** times with Wash Buffer as described in the Plate Washing section.

### D. Streptavidin-HRP Solution Incubation

- Prepare Streptavidin-HRP Solution **immediately before use**. Do not prepare more Streptavidin-HRP than required. Do not store prepared Streptavidin-HRP Solution.
  - Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
  - If using a multichannel pipettor, **use new reagent reservoir and 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 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.
  3. For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.

4. Add 100µL of prepared Streptavidin-HRP Solution to each well.
5. 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.
6. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing section.

### E. 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 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 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. Stop the reaction by adding 100µL of Stop Solution to each well.

### F. Absorbance Measurement

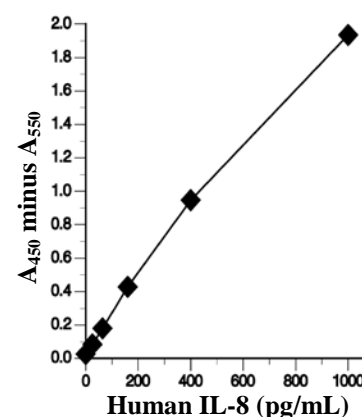
**Note:** Evaluate the plate within 30 minutes of stopping the reaction.

1. 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 the absorbance at 450nm only. Omitting the 550nm measurement will result in higher absorbance values.

### G. Calculation of Results

- Use the standard curve to determine human IL-8 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-8 concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the IL-8 amount in each sample by interpolating from the absorbance value (Y axis) to IL-8 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-8 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:** < 2pg/mL

The sensitivity or Lower Limit of Detection (LLD)<sup>1</sup> 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%

Inter-assay CV: < 10%

**Specificity:** This ELISA is specific for the measurement of natural and recombinant human IL-8 and has been shown to recognize IL-8 derived from stimulated human umbilical vein endothelial cells. It does not cross-react with human PF-4, GRO $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-12, TNF $\alpha$ , IFN $\gamma$ , or GM-CSF.

**Calibration:** The ELISA standard is calibrated to NIBSC standard lot 89/520. One (1) pg = 1.3 NIBSC pg = 0.0013 NIBSC units

**Expected Values:** The mean level of human IL-8 in 11 normal serum samples was 8.6pg/mL ranging from 1.2 to 16.7pg/mL. The mean level of human IL-8 in 11 normal plasma samples was 2.0pg/mL ranging from 0 to 8.1pg/mL. The mean level of human IL-8 in five normal urine samples was 45.3pg/mL ranging from 0 to 204pg/mL.

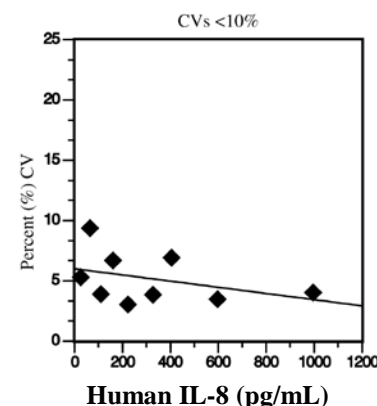
**Precision:** The intra-assay coefficient of variation is plotted against IL-8 concentration (pg/mL). The points represent samples Biotinylated Antibody Reagent evaluated in replicates of four in two different kit lots.

#### Human IL-8 ELISA Precision Profile

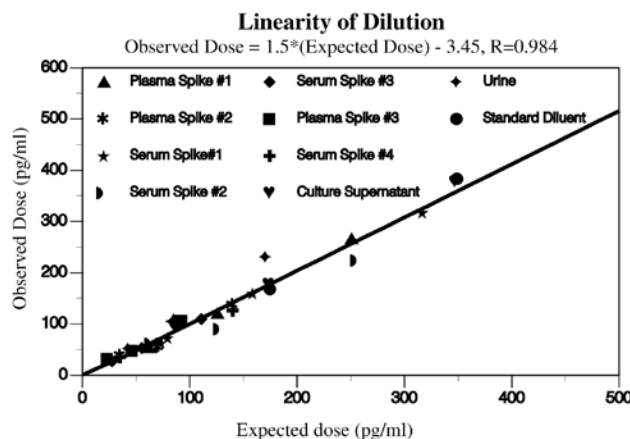
**Recovery:** Various levels of recombinant human IL-8 were spiked into normal human, serum, plasma and urine samples, and a control buffer. Mean recoveries are as follows:

<b>Recombinant Control Value:</b>	<b>135pg/mL</b>	<b>216pg/mL</b>	<b>724pg/mL</b>
Mean Serum Recovery	103%	101%	94%
Mean Plasma Recovery	81%	82%	80%

<b>Recombinant Control Value:</b>	<b>293pg/mL</b>	<b>682pg/mL</b>
Mean Urine Recovery	117%	124%



**Linearity of Dilution:** Ten different positive samples were serially diluted. The dilutions were evaluated in the ELISA and the “observed” doses are plotted against the “expected” doses.



#### Reference

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

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## Data Templates

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