INSTRUCTIONS

Thermo SCIENTIFIC

Human IL-16 ELISA Kit

EHIL16 EHIL162 EHIL165

1381.4

Number	**Description**
EHIL16	**Human Interleukin-16 (IL-16) ELISA Kit,** sufficient reagents for 96 determinations
EHIL162	**Human Interleukin-16 (IL-16) ELISA Kit,** sufficient reagents for 2 x 96 determinations
EHIL165	**Human Interleukin-16 (IL-16) ELISA Kit,** sufficient reagents for 5 x 96 determinations

Kit Contents	**EHIL16**	**EHIL162**	**EHIL165**
Anti-human IL-16 Pre-coated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Human IL-16 Standard	2 vials	4 vials	10 vials
Standard Diluent, contains 0.1% NaN_3	26mL	2 x 26mL	5 x 26mL
Biotinylated Antibody Reagent, contains 0.1% NaN_3	8mL	2 x 8mL	5 x 8mL
30X Wash Buffer	50mL	2 x 50mL	5 x 50mL
Streptavidin-HRP Concentrate	75µL	2 x 75µL	5 x 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 x 14mL	5 x 14mL
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	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.

Table of Contents

Introduction 1
Procedure Summary 2
Additional Materials Required 2
Precautions 2
Sample Preparation 3
Reagent Preparation 4
Assay Procedure 4
Performance Characteristics 6
Cited Reference 7
Data Templates 8

Introduction

The Thermo Scientific™ Human IL-16 ELISA Kit measures human IL-16 in culture supernatants; EDTA, heparin and sodium citrate plasma; and serum.

Procedure Summary

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

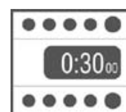
2. Cover plate and incubate at room temperature (20-25°C) for 1 hour. Do not wash or empty the plate.

3. Add 50µL of Biotinylated Antibody Reagent to each well.

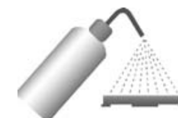
4. Cover plate and incubate at room temperature for 1 hour.

5. Wash plate THREE times.

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



7. Cover plate and incubate at room temperature for 30 minutes.



8. Wash plate THREE times.

9. Add 100µL of TMB Substrate to each well.

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

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

12. Measure absorbance on a plate reader at 450nm minus 550nm. Calculate results using graph paper or curve-fitting statistical software.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL
- Plastic pipettes to deliver 5-15mL
- Ultrapure water
- 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 reservoir, 4 each
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- Microcentrifuge 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 ELISA**.
- Review the 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 samples 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.
- 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 kit components. 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 the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

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, heparin or sodium citrate plasma; or culture supernatants may be tested in this ELISA.
- 50µL per well of serum, plasma 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 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-16 concentration possibly exceeds the highest point of the standard curve (i.e., 2000pg/mL), prepare one or more five-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 five-fold dilution is prepared by adding 50µL of test sample to 200µL 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 2L container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. Add the entire contents of the 30X Wash Buffer bottle (50mL) 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.

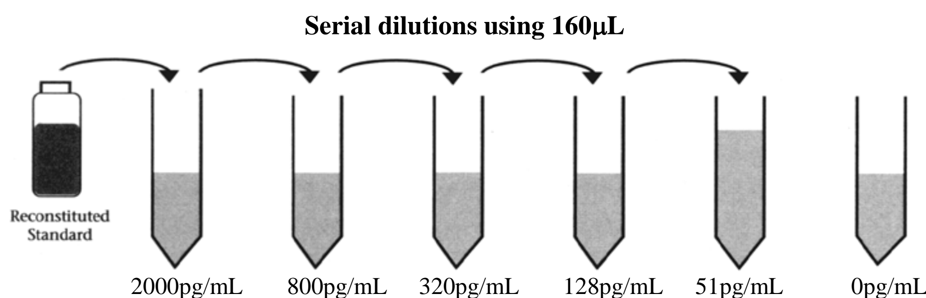
   Note: Wash Buffer must be at room temperature before use in this assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

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. 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.
2. Label six tubes, one for each standard curve point: 2000, 800, 320, 128, 51, and 0pg/mL, then prepare 1:2.5 serial dilutions for the standard curve as follows:
3. Pipette 240µL of Standard Diluent into each tube.
4. Pipette 160µL of the reconstituted standard into the first tube (i.e., 2000pg/mL) and mix.
5. Pipette 160µL of this dilution into the second tube (i.e., 800pg/mL) and mix.
6. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points. These concentrations, 2000pg/mL, 800pg/mL, 320pg/mL, 128pg/mL, 51pg/mL, and 0pg/mL are the standard curve.

Serial dilutions using 160µL



Assay Procedure

A. Sample and Biotinylated Antibody Reagent Incubation

- **(PP)** Determine 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), 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 reconstituted standards or test samples in duplicate to each well.

   Note: If the human IL-16 concentration in any test sample possibly exceeds the highest point on the standard curve, 2000pg/mL, see Sample Preparation–Sample Dilution Section.

2. Add 50µL of Standard Diluent to all wells that do not contain standards or samples.
3. 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 one (1) hour at room temperature, 20-25°C.

   Note: Do NOT empty or wash the plate.
4. Add 50µL of Biotinylated Antibody Reagent to each well containing standards or samples. Take care not to cross-contaminate the standards or samples in the plate. Mix well by gently tapping the plate several times.
5. 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 one (1) hour at room temperature.
6. Carefully remove adhesive plate cover. Wash plate THREE times with Wash Buffer as 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 wash 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 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 a 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.
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.

   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 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 (section B).

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

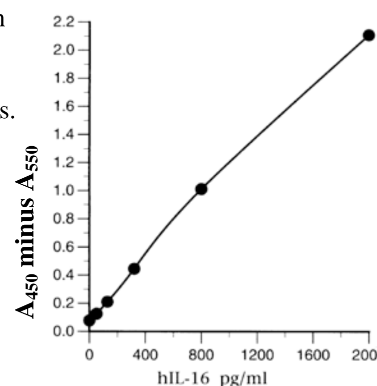
Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure the 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 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-16 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-16 concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the human IL-16 amount in each sample by interpolating from the absorbance value (Y axis) to human IL-16 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 IL-16 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: < 8pg/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.

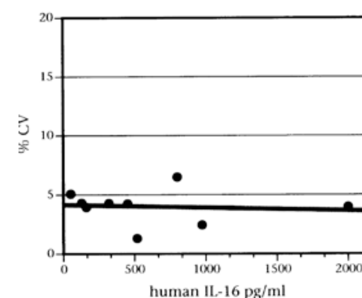
Assay Range: 0-2000pg/mL
Suggested standard curve points are 2000, 800, 320, 128, 51, and 0pg/mL.

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

Specificity This ELISA is specific for the measurement of natural and recombinant human IL-16. The following human cytokines and chemokines do not interfere with or cross-react in this ELISA: Eotaxin, G-CSF, GM-CSF, GROα, GROβ, IFNα, IFNγ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-17, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1α, MIP-1β, TNFα, TNFβ, or RANTES.

Calibration: This ELISA is calibrated to an internal human IL-16 reference standard.

Precision: The intra-assay coefficient of variation is plotted against human IL-16 concentration (pg/mL). The points represent samples evaluated in replicates of four in four different kit lots.



Expected Values: Serum and plasma samples collected from apparently healthy individuals are evaluated in this assay. The levels of human IL-16 in these samples are reported in Table 1.

Table 1. Human IL-16 levels from apparently healthy individuals.

Sample Type	Average	Range
Serum (n=35)	518pg/mL	32-2739pg/mL
EDTA plasma (n=35)	1426pg/mL	12-6331pg/mL
Citrate plasma (n=8)	264pg/mL	19-1631pg/mL
Heparin plasma (n=8)	225pg/mL	97-480pg/mL

Spike and Recovery: Recovery in serum and plasma collected with various anticoagulants is determined by spiking 1000pg/mL recombinant human IL-16 into matched serum and plasma samples collected from five apparently healthy individuals and a Standard Diluent control buffer. Mean recoveries are reported in Table 2.

Table 2. Human IL-16 recovery in serum and plasma from apparently healthy individuals.

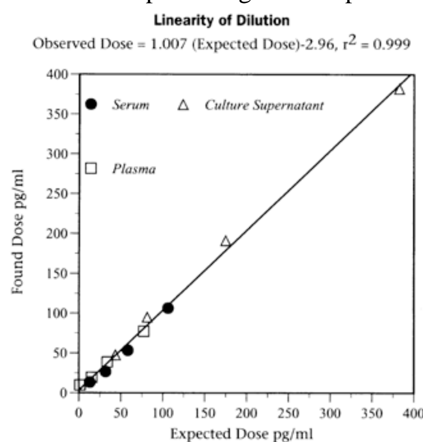
Sample Type	Average	Range
Serum (n=5)	89%	80-98%
EDTA plasma (n=5)	80%	65-89%
Citrate plasma (n=5)	82%	67-93%
Heparin plasma (n=5)	85%	78-91%

Recovery across the standard curve range is determined by spiking various levels of recombinant human IL-16 into serum and plasma samples collected from apparently healthy individuals, and a Standard Diluent control buffer. Mean recoveries are reported in Table 3.

Table 3. Human IL-16 recovery across the standard curve range.

Spike Level	500pg/mL	1000pg/mL	1500pg/mL
EDTA plasma (n=5)	84%	85%	85%
Serum (n=5)	90%	76%	97%

Linearity of Dilution: Dilution linearity is determined by serially diluting serum, plasma, and culture supernatant samples. The dilutions are evaluated in the ELISA and "found" doses are plotted against "expected" doses.



Cited Reference

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

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.
NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NON-CONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2014 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.

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												
G												
H												

Pierce Biotechnology
3747 N. Meridian Road
PO Box 117
Rockford, IL 61105 USA
(815) 968-0747
(815) 968-7316 fax
www.thermoscientific.com/pierce