Human IL-17F (homodimer) Uncoated ELISA

Catalog Number: 88-7478
Also known as: Interleukin-17F, IL-17FF

Product Information

- **Contents:** Human IL-17F (homodimer) Uncoated ELISA
  - Catalog Number: 88-7478
  - Sensitivity: 16 pg/mL
  - Standard Curve Range: 16 - 2000 pg/mL

- **Temperature Limitation:** Store at 2-8°C except standard which should be stored at less than or equal to -70°C.

- **Batch Code:** Refer to vial
- **Use By:** Refer to vial

Description

This human IL-17F Uncoated ELISA set (with or without high-affinity binding microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA reagent set is specifically engineered for accurate and precise measurement of human IL-17F homodimer protein levels from samples including serum, plasma and supernatants from cell cultures. The assay demonstrates parallelism in measuring recombinant and native human IL-17F proteins and a standard curve range of 16 to 2000 pg/mL. This human IL-17F ELISA Uncoated is very specific to IL-17F homodimer. No crossreactivity to the IL-17A homodimer is observed and 0.24% crossreactivity to recombinant human IL-17AF heterodimer when spiked in excess (100ng/ml).

IL-17F is a 37kD homodimer of the IL-17 family and a signature Th17 marker. Of all the six IL-17 family members, IL-17F and IL-17A share the strongest homology (50% amino acid identity), and the two genes are located in the same chromosomal region. It has been reported that the secreted IL-17A, IL-17F and IL-17AF can be detected in activated human peripheral blood lymphocytes, specifically by activated human CD4+ T cells. IL-17F has been reported to be involved in inflammatory diseases. IL-17F expression has been observed in tissue samples from various autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, and asthma.

Components

- **Capture Antibody:** Pre-titrated, purified antibody
- **Detection Antibody:** Pre-titrated, biotin-conjugated antibody
- **Standard:** Recombinant cytokine for generating standard curve and calibrating samples
- **Coating Buffer:** 10X Carbonate ELISA Coating Buffer
- **Assay Diluent:** 5X concentrated
- **Detection enzyme:** Pre-titrated Avidin-HRP
- **Substrate Solution:** Tetramethylbenzidine (TMB) Substrate Solution
- **Certificate of Analysis:** Lot-specific instructions for dilution of antibodies and standards
- **96 Well Plate:** NUNC Maxisorp flat-bottom (included with product Cat. #’s ending in suffixes -22, -44, -76, -86)
Human IL-17F (homodimer) Uncoated ELISA
Catalog Number: 88-7478
Also known as: Interleukin-17F, IL-17FF

Applications Reported
Human IL-17F (homodimer) Uncoated ELISA has been reported for use in ELISA.

References
Chang SH, Dong C. A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. Cell Res. 2007 May;17(5):435-40.


Related Products
14-8479 Human IL-17F Recombinant Protein
88-7117 Human IL-17AF (heterodimer) Uncoated ELISA
88-7176 Human IL-17A (homodimer) Uncoated ELISA
Enzyme Linked Immunosorbent Assay (ELISA)

Protocol: Uncoated ELISA
The following protocol is a general guideline for the Uncoated Sets

Materials Provided
- Please refer to the Certificate of Analysis (C of A) for components

Other Materials Needed
- Buffers*
  - Wash Buffer: 1x PBS, 0.05% Tween-20 (or Thermo Fisher ELISA Wash Buffer Powder, Cat. No. 00-0400)
  - Stop Solution: 1M H₃PO₄ (recommended) or 2N H₂SO₄
- Pipettes
- Refrigerator & frost-free -20°C freezer
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp™)
  **NOTE:** The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp (Cat. No. 44-2404) 96-well plates.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer (highly recommended)

**NOTE:** To ensure optimal results from this Uncoated ELISA Set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Time Requirements
- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

Experimental Procedure
1. Coat Corning Costar 9018 (or Nunc Maxisorp™) ELISA plate with 100 µL/well of capture antibody in 1X Coating Buffer (dilute as noted on C of A, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with >250 µL/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X ELISA/ELISPOT Diluent with 4 parts DI water*. Block wells with 200 µL/well of 1X ELISA/ELISPOT Diluent. Incubate at room temperature for 1 hour.
4. Optional: Aspirate and wash at least once with Wash Buffer.
5. Using 1X ELISA/ELISPOT Diluent *, dilute standards as noted on the C of A to prepare the top concentration of the standard. Add 100 µL/well of top standard concentration to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve for a total of 8 points. Add 100 µL/well of your samples to the appropriate wells. Seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
7. Add 100 µL/well of detection antibody diluted in 1X ELISA/ELISPOT Diluent * (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
9. Add 100 µL/well of Avidin-HRP* diluted in 1X ELISA/ELISPOT Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 5-7 washes**.
11. Add 100 µL/well of 1X TMB Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µL of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

**NOTES:**
* Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.
**The number of washes in the protocol was adapted to an automatic plate washer. This can be decreased when using other methods but should be tested empirically. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes.
Quick Guide: Standard Calibration

The following table indicates the protein standard contained in the Uncoated kit is calibrated against NIBSC standards.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>ng of eB standard</th>
<th>ng of NIBSC standard</th>
<th>U of NIBSC standard</th>
<th>NIBSC Lot #</th>
</tr>
</thead>
<tbody>
<tr>
<td>hIL-2</td>
<td>1</td>
<td>1.1</td>
<td>14.6</td>
<td>86/564</td>
</tr>
<tr>
<td>hIL-4</td>
<td>1</td>
<td>2.2</td>
<td>22</td>
<td>88/656</td>
</tr>
<tr>
<td>hIL-5</td>
<td>1</td>
<td>2.2</td>
<td>22</td>
<td>90/586</td>
</tr>
<tr>
<td>hIL-6</td>
<td>1</td>
<td>1.7</td>
<td>170</td>
<td>89/548</td>
</tr>
<tr>
<td>hIL-8</td>
<td>1</td>
<td>1.8</td>
<td>180</td>
<td>89/520</td>
</tr>
<tr>
<td>hIL-10</td>
<td>1</td>
<td>0.8</td>
<td>4</td>
<td>93/722</td>
</tr>
<tr>
<td>hIL-12</td>
<td>1</td>
<td>0.8</td>
<td>8</td>
<td>95/544</td>
</tr>
<tr>
<td>hIL-17A</td>
<td>1</td>
<td>0.9</td>
<td>9000</td>
<td>01/420</td>
</tr>
<tr>
<td>hIFN-g</td>
<td>1</td>
<td>1.1</td>
<td>22</td>
<td>87/586</td>
</tr>
<tr>
<td>hTNF-a</td>
<td>1</td>
<td>0.9</td>
<td>36</td>
<td>87/650</td>
</tr>
<tr>
<td>mIL-2</td>
<td>1</td>
<td>3.1</td>
<td>310</td>
<td>93/566</td>
</tr>
<tr>
<td>mIL-4</td>
<td>1</td>
<td>3</td>
<td>30</td>
<td>91/656</td>
</tr>
<tr>
<td>mIL-6</td>
<td>1</td>
<td>8.5</td>
<td>850</td>
<td>93/730</td>
</tr>
<tr>
<td>mILN-g*</td>
<td>1</td>
<td>1.7</td>
<td>4.5</td>
<td>Gg02-901-533</td>
</tr>
<tr>
<td>mTNF-a</td>
<td>1</td>
<td>1.7</td>
<td>340</td>
<td>88/532</td>
</tr>
</tbody>
</table>

* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)

ELISA Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possibility</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. High background</td>
<td>1. Improper and inefficient washing</td>
<td>1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed.</td>
</tr>
<tr>
<td></td>
<td>2. Cross contamination from other specimens or positive control</td>
<td>2. Repeat ELISA being careful when washing and pipetting.</td>
</tr>
<tr>
<td></td>
<td>4. Incorrect dilutions, e.g., conjugate concentration was too high</td>
<td>4. Repeat using correct dilutions.</td>
</tr>
<tr>
<td>B. No signal</td>
<td>1. Improper, low protein binding capacity plates were used</td>
<td>1. Repeat ELISA using recommended high binding capacity plates.</td>
</tr>
<tr>
<td></td>
<td>2. Wrong substrate was used</td>
<td>2. Repeat ELISA using the correct substrate</td>
</tr>
<tr>
<td></td>
<td>3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity</td>
<td>3. Repeat ELISA making no enzyme inhibitor is present in any buffers.</td>
</tr>
<tr>
<td></td>
<td>4. Coated capture antibody in ELISA/ELISPOT Diluent rather than Coating Buffer</td>
<td>4. Repeat ELISA using Coating Buffer contained in the set as the diluent for the capture antibody.</td>
</tr>
</tbody>
</table>
# ELISA Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possibility</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Very weak signal</td>
<td>1. Improper and inefficient washing</td>
<td>1. Make sure washing procedure is done correctly, with a soak time.</td>
</tr>
<tr>
<td></td>
<td>2. Incorrect dilutions of standard</td>
<td>2. Follow recommendations of standard preparation exactly as written on the C of A.</td>
</tr>
<tr>
<td></td>
<td>3. Insufficient incubation time</td>
<td>3. Repeat ELISA following the protocol carefully for each step.</td>
</tr>
<tr>
<td></td>
<td>4. Incorrect storage of reagents</td>
<td>4. Store reagents at the correct temperature as indicated on the Technical Data Sheet. Freezing certain components will severely impact results. Do not re-use the standards.</td>
</tr>
<tr>
<td></td>
<td>5. Wrong filter in ELISA reader was used</td>
<td>5. Use the correct wavelength setting.</td>
</tr>
<tr>
<td></td>
<td>6. Wrong plate used</td>
<td>6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates.</td>
</tr>
<tr>
<td>D. Variation amongst replicates</td>
<td>1. Improper and inefficient washing</td>
<td>1. Make sure washing procedure is done correctly; see C of A. Edge effects can be avoided by moving samples and standards from the edge of the plate.</td>
</tr>
<tr>
<td></td>
<td>2. Poor mixing of samples</td>
<td>2. Mix samples and reagents gently and equilibrate to proper temperature.</td>
</tr>
<tr>
<td></td>
<td>3. Plates not clean</td>
<td>3. Plates should be wiped on bottom before measuring absorbance</td>
</tr>
<tr>
<td></td>
<td>4. Reagents have expired</td>
<td>4. Order a new Ready-Set-Go ELISA.</td>
</tr>
</tbody>
</table>
Documentation and support

Customer and technical support
Visit thermofisher.com/support for the latest in services and support, including:
- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)
Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty
Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at thermofisher.com/support.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288
©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. All other trademarks are properties of their respective owners.