Human IL-1 beta Uncoated ELISA
Catalog Number: 88-7261
Also known as: Interleukin-1 beta

Product Information
Contents: Human IL-1 beta Uncoated ELISA
Catalog Number: 88-7261
Sensitivity: 2 pg/mL
Standard Curve Range: 2 - 150 pg/mL
Temperature Limitation: Store at 2-8°C.
Batch Code: Refer to vial
Use By: Refer to vial

Description
This Human IL-1 beta Uncoated ELISA Set contains the necessary reagents, standards, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement protein levels from samples including serum, plasma, and supernatants from cell cultures. This ELISA will recognize the cleaved mature form and uncleaved pro-form of Human IL-1 beta. This second generation kit has increased sensitivity with a range from 2-150 pg/mL.

IL-1 beta is a proinflammatory cytokine expressed by monocytes, macrophages, and dendritic cells. It is synthesized in response to inflammatory stimuli as a 31 kDa inactive pro-form that accumulates in the cytosol. Cleavage of pro-IL-1 beta into the active 17 kDa protein requires the activation of inflamasomes, which are multi-protein complexes that respond to pathogens, stress conditions, and other danger signals. Inflammasome activation triggers the processing of the caspase-1 precursor into its active form, which in turn cleaves pro-IL-1 beta. IL-1 beta lacks a signal sequence peptide for classical ER/Golgi pathway and is instead secreted alongside caspase-1 via an alternate and incompletely understood mechanism. IL-1 beta signals through two receptors, IL-1RI and IL-1RII, both of which are shared with IL-1 alpha. These cytokines play important roles in innate host defense by triggering the production of other proinflammatory cytokines in target cells and initiating acute-phase responses. Their activity can be moderated by IL-1 Receptor Antagonist (IL-1RA), a protein produced by many cell types that blocks receptor binding through competitive inhibition. Elevated levels of IL-1 beta have been associated with many chronic inflammatory conditions, giving IL-1RA or IL-1 beta neutralizing antibodies potential therapeutical value.

Components
Capture Antibody: Pre-titrated, purified antibody
Detection Antibody: Pre-titrated, biotin-conjugated antibody
Standard: Recombinant protein for generating standard curve and calibrating samples
10X Coating Buffer : Buffer for plating the Capture Antibody
5X ELISA/ELISPOT Diluent : Buffer for blocking and diluting the Detection Antibody and Enzyme
Enzyme: Pre-titrated Avidin-HRP
Substrate: 1X TMB Solution
Certificate of Analysis: Lot-specific instructions for dilution of antibodies and standards
Human IL-1 beta Uncoated ELISA

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96 Well Plate: Corning Costar 9018 (included with product Cat. #’s ending in suffixes -22, -76, -86)

Applications Reported
This ELISA set is for the quantitative detection of human IL-1 beta in serum, plasma, and tissue culture supernatant samples.

Applications Tested
This assay has been validated for the detection of endogenous human IL-1 beta with supernatant collected from LPS-stimulated cultures of normal peripheral blood monocytes. Lower levels of IL-1 beta are observed in unstimulated cultures.

This assay was evaluated for specificity on a panel of 72 recombinant cytokines at 100 ng/mL. No significant crossreactivity was observed, including to recombinant mouse IL-1 beta.

References


Related Products
00-0400 ELISA Wash Buffer - 10 x 1L Packets
00-4201 1X TMB Solution
00-4202 5X ELISA/ELISPOT Diluent
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>mIFN-g*</td>
<td>1</td>
<td></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 in 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

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- Worldwide contact telephone numbers
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  - 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
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