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Introduction

Interleukin-1β (IL-1β) is a potent immunomodulator which mediates a wide range of immune and inflammatory responses including the activation of B and T cells.\textsuperscript{1,2} The mature form of human IL-1β is a 17 kD protein containing 153 amino acid residues, representing amino acids A117 through S269 of the IL-1β precursor protein.

The BD OptEIA\textsuperscript{TM} Human IL-1β ELISA Kit is for the quantitative determination of Human IL-1β in serum, plasma, and cell culture supernatant.

Principle of the Test

The BD OptEIA test is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for human IL-1β coated on a 96-well plate. Standards and samples are added to the wells, and any IL-1β present binds to the immobilized antibody. The wells are washed and biotinylated anti-human IL-1β antibody is added, producing an antibody-antigen-antibody “sandwich”. After a second wash, streptavidin-horseradish peroxidase is added. The wells are again washed and TMB substrate solution is added, which produces a blue color in direct proportion to the amount of IL-1β present in the initial sample. The Stop Solution changes the color from blue to yellow, and the wells are read at 450 nm.
Reagents Provided

Antibody Coated Wells: 2 plates of 96 breakable wells (12 strips × 8 wells) coated with anti-human IL-1β monoclonal antibody

Detection Antibody: 30 mL of biotinylated anti-human IL-1β polyclonal antibody with 0.015% ProClin™- 150 as preservative

Standards: 4 vials lyophilized recombinant human IL-1β

Enzyme Concentrate (250×): 150 μL of 250× concentrated Streptavidin-horseradish peroxidase conjugate with BSA* and ProClin™- 150 as preservative

Enzyme Diluent: 30 mL of a buffered protein base with ProClin™-150 as preservative

ELISA Dilution reagent: 30 mL of a buffered protein base with FBS* and 0.09% sodium azide as preservative

Wash Concentrate (20×): 100 mL of 20× concentrated detergent solution with ProClin™- 150 as preservative

TMB One-Step Substrate Reagent: 30 mL of 3,3′,5,5′-tetramethylbenzidine (TMB) in buffered solution

Stop Solution: 13 mL of 1 M phosphoric acid

Plate Sealers: 4 sheets with adhesive backing

*Source of all serum proteins is from USDA inspected abattoirs located in the United States

Materials Required but not Provided

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 50 μL and 100 μL volume
- Adjustable 1 mL, 5 mL, 10 mL, 25 mL pipettes for reagent preparation
- Deionized or distilled water
- Wash bottle or automated microplate washer
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard dilutions
- Laboratory timer
- Absorbent paper

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Storage Information

1. Store unopened kit at 2 - 8°C. Do not use kit after expiration date.

2. Before use, bring all reagents to room temperature (18 - 25°C). Immediately after use, return to proper storage conditions.

3. Lyophilized standards are stable until kit expiration date. After reconstitution, use freshly reconstituted standard within 12 hours (stored at 2 - 8°C).

Warnings and Precautions

1. Reagents that contain preservatives may be toxic if they are ingested, inhaled, or come in contact with skin.

2. Avoid contact of skin, eyes, or clothing with Stop Solution or Substrate Reagents.

3. Handle all serum and plasma specimens in accordance with NCCLS guidelines for preventing transmission of blood-borne infections.

4. Warning

Wash Concentrate (20X) (component 51-9003738) contains 0.002% (w/w), Enzyme Diluent (component 51-2718KD) contains 0.003% (w/w), Human IL-1β Lyophilized Standard (component 51-9004474) contains 0.03% (w/w) and Detection Antibody Biotin Anti-Human IL-1β (component 51-9002712) contains 0.003% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).

Hazard statements

May cause an allergic skin reaction.

Precautionary statements

Wear protective gloves / eye protection.

Wear protective clothing.

Avoid breathing mist/vapours/spray.

If skin irritation or rash occurs: Get medical advice/attention.

IF ON SKIN: Wash with plenty of water.

Dispose of contents/container in accordance with local/regional/national/international regulations.

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5. **Danger**

Stop Solution (component 51-2608KC) contains 15.23% phosphoric acid (w/w).

**Hazard statements**

*Causes severe skin burns and eye damage.*

**Precautionary statements**

*Wear protective gloves / eye protection.*

*Wear protective clothing.*

*IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.*

*IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.*

*Continue rinsing.*

*IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.*

*Dispose of contents/container in accordance with local/regional/national/international regulations.*

**Specimen Collection and Handling**

Specimens should be clear, non-hemolyzed and non-lipemic. Specimens with expected values greater than 125 pg/mL should be diluted with Standard/Sample Diluent prior to running the assay.

**Cell culture supernatants:** Remove any particulate material by centrifugation and assay immediately or store samples at \( \leq -20^\circ\text{C} \). Avoid repeated freeze-thaw cycles.

**Serum:** Use a serum tube (eg, BD Vacutainer®, Cat. No. 366430) and allow samples to clot for 30 minutes, then centrifuge for 10 minutes at 1000 \( \times \) g. Remove serum and assay immediately or store samples at \( \leq -20^\circ\text{C} \). Avoid repeated freeze-thaw cycles.

**Plasma:** Collect plasma using heparin, or citrate as anticoagulant. Centrifuge for 10 minutes at 1000 \( \times \) g within 30 minutes of collection. Assay immediately or store samples at \( \leq -20^\circ\text{C} \). Avoid repeated freeze-thaw cycles.
Reagent Preparation

1. Bring all reagents to room temperature (18 - 25°C) before use.

2. Standards

   a. Reconstitute 1 vial lyophilized Standard with required volume (noted on vial label) of ELISA dilution buffer to prepare a 125 pg/mL stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Vortex to mix.

   b. Add 300 µL ELISA dilution buffer to 6 tubes. Label as 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, 7.8 pg/mL, 3.9 pg/mL, and 1.95 pg/mL.

   c. Perform serial dilutions by adding 300 µL of each standard to the next tube and vortexing between each transfer. The undiluted standard serves as the high standard (125 pg/mL). The ELISA dilution buffer serves as the zero standard (0 pg/mL).

3. Wash Buffer

   Note: If the Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute required quantity of 20× Wash Concentrate with deionized or distilled water, mix. (To prepare 2,000 mL, add 100 mL Wash Concentrate to 1,900 mL water. At least 500 mL solution should be prepared for a full 96-well plate).

4. TMB One-Step Substrate Reagent

   Add required volume of TMB One-Step Substrate Reagent to a clean tube or reservoir. Pipette out from the tube/ reservoir instead of directly from the bottle, to prevent contamination. Avoid prolonged exposure to light or contact with metal, air, or extreme temperature as color may develop.

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Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) prior to use. It is recommended that all standards and samples be run in duplicate. A standard curve is required in each assay run.

2. Remove required quantity of test strips/wells, place in well holder.
   
   **Note:** Wells are provided in breakable 8-well strips. Strips may be “broken” into individual wells, replaced in well holder, and assayed. Return any unused wells to sealed pouch for 2 - 8°C storage.

3. Pipette 100 µL of each standard (see *Reagent Preparation*, step 2) and sample into appropriate wells. Cover wells with Plate Sealer and incubate for 2 hours at room temperature.

4. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µL/well prepared Wash Buffer (see *Reagent Preparation*, step 3), followed by decanting/aspirating. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove any residual buffer. Complete removal of liquid is required for proper performance.

5. Add 100 µL of Detection Antibody to each well. Cover wells with Plate Sealer and incubate for 1 hour at room temperature.

6. Prepare Enzyme Working Reagent. Pipette required volume of Enzyme Diluent into a clean tube or flask. Add in required quantity of Enzyme Concentrate (250×), vortex or mix well. For a full 96-well plate, add 48 µL of Enzyme Concentrate into 12 mL of Enzyme Diluent.

7. Wash wells as in Step 5.

8. Add 100 µL of Enzyme Working Reagent (see step 7 above) to each well. Cover wells with Plate Sealer and incubate for 30 minutes at room temperature.

9. Wash wells as in Step 5, but a total of 7 times.
   
   **Note:** In this final wash step, soak wells in wash buffer for 30 seconds to 1 minute for each wash. Thorough washing at this step is very important.

10. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate plate (without Plate Sealer) for 30 minutes at room temperature in the dark.

11. Add 50 µL of Stop Solution to each well.

12. Read absorbance at 450 nm within 30 minutes of stopping reaction.
   If wavelength correction is available, subtract the optical density readings at 570 nm from readings at 450 nm.

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Assay Procedure Summary

1. Add 100 μL standard or sample to each well. Incubate 2 hours at room temperature.
2. Aspirate and wash 5 times.
3. Add 100 μL Detection Antibody to each well. Incubate 1 hour at room temperature.
4. Aspirate and wash 5 times.
5. Add 100 μL Enzyme Working Reagent to each well. Incubate 30 minutes at room temperature.
6. Aspirate and wash/soak 7 times.
7. Add 100 μL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
8. Add 50 μL Stop Solution to each well. Read at 450 nm within 30 minutes. λ correction 570 nm.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance (ie, plate background) from each.

Plot the standard curve on log-log graph paper, with IL-1β concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points.

To determine the IL-1β concentration of the unknowns, find the unknowns’ mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the IL-1β concentration. If samples were diluted, multiply the interpolated IL-1β concentration by the dilution factor.

Computer-based curve-fitting statistical software may also be employed.

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Typical Data
This standard curve is for demonstration only. A standard curve must be run with each assay.

<table>
<thead>
<tr>
<th>Concentration (pg/mL)</th>
<th>OD1</th>
<th>OD2</th>
<th>OD3</th>
<th>Mean</th>
<th>Zero Standard Subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.008</td>
<td>0.009</td>
<td>0.009</td>
<td>0.009</td>
<td>0.000</td>
</tr>
<tr>
<td>1.953</td>
<td>0.068</td>
<td>0.069</td>
<td>0.070</td>
<td>0.069</td>
<td>0.060</td>
</tr>
<tr>
<td>3.906</td>
<td>0.125</td>
<td>0.128</td>
<td>0.128</td>
<td>0.127</td>
<td>0.118</td>
</tr>
<tr>
<td>7.813</td>
<td>0.243</td>
<td>0.252</td>
<td>0.258</td>
<td>0.251</td>
<td>0.242</td>
</tr>
<tr>
<td>15.625</td>
<td>0.472</td>
<td>0.490</td>
<td>0.469</td>
<td>0.477</td>
<td>0.468</td>
</tr>
<tr>
<td>31.250</td>
<td>0.854</td>
<td>0.847</td>
<td>0.865</td>
<td>0.855</td>
<td>0.846</td>
</tr>
<tr>
<td>62.500</td>
<td>1.560</td>
<td>1.640</td>
<td>1.600</td>
<td>1.600</td>
<td>1.591</td>
</tr>
<tr>
<td>125.000</td>
<td>3.117</td>
<td>3.088</td>
<td>3.018</td>
<td>3.074</td>
<td>3.065</td>
</tr>
</tbody>
</table>

Limitations of the Procedure
1. This kit is intended for use as an integral unit. Do not mix reagents from different kit lots. Reagents from other manufacturers/other available clones should not be used in this kit.

2. Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.

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Performance

Limit of Detection
The minimum detectable dose of human IL-1β was determined to be 0.8 pg/mL. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standard.

Recovery
Three different levels of human IL-1β were spiked into the samples each of various matrices. Results are compared with same amounts of human IL-1β spiked into Standard Diluent, as follows:

<table>
<thead>
<tr>
<th>Spike Concentration (ng/mL)</th>
<th>Average % Recovery</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>101.83</td>
<td>98.5 - 107.8</td>
</tr>
<tr>
<td>62.5</td>
<td>92.85</td>
<td>88.3 - 97.4</td>
</tr>
<tr>
<td>31.25</td>
<td>90.45</td>
<td>86.8 - 95.8</td>
</tr>
<tr>
<td>Plasma (n=6) in NA Citrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>102.35</td>
<td>98.9 - 105.2</td>
</tr>
<tr>
<td>62.5</td>
<td>89.65</td>
<td>78.6 - 103.8</td>
</tr>
<tr>
<td>31.25</td>
<td>89.52</td>
<td>70.4 - 99.9</td>
</tr>
<tr>
<td>Plasma (n=4) in Heparin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>103.73</td>
<td>99.4 - 109.3</td>
</tr>
<tr>
<td>62.5</td>
<td>92.93</td>
<td>83.5 - 96.6</td>
</tr>
<tr>
<td>31.25</td>
<td>95.95</td>
<td>92.2 - 99.2</td>
</tr>
<tr>
<td>Cell Culture Media (n=2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>107.30</td>
<td>107.1 - 107.5</td>
</tr>
<tr>
<td>62.5</td>
<td>92.65</td>
<td>91.2 - 94.1</td>
</tr>
<tr>
<td>31.25</td>
<td>91.65</td>
<td>91.1 - 92.2</td>
</tr>
</tbody>
</table>

Linearity
Samples spiked with high concentrations of human IL-1β were serially diluted with Standard Diluent and run in the BD OptEIA human IL-1β Kit. Results are as follows:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Cell Culture Media (n=2)</th>
<th>Serum (n=6) in Heparin</th>
<th>Plasma (n=4) in Na Citrate</th>
<th>Plasma (n=6) in Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>Average % of Expected Range</td>
<td>98.00 95.9 - 101.1</td>
<td>100.52 93.5 - 104.0</td>
<td>100.63 97.0 - 102.5</td>
</tr>
<tr>
<td>1:4</td>
<td>Average % of Expected Range</td>
<td>94.80 94.5 - 95.1</td>
<td>98.72 93.2 - 101.6</td>
<td>101.45 96.6 - 105.3</td>
</tr>
<tr>
<td>1:8</td>
<td>Average % of Expected Range</td>
<td>85.50 84.2 - 86.8</td>
<td>93.43 86.0 - 100.1</td>
<td>92.03 87.8 - 95.8</td>
</tr>
<tr>
<td>1:16</td>
<td>Average % of Expected Range</td>
<td>79.80 79.5 - 80.1</td>
<td>79.33 75.2 - 82.1</td>
<td>82.70 76.9 - 87.4</td>
</tr>
</tbody>
</table>

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Specificity

Cross-Reactivity: The following factors were tested in the BD OptEIA™ Human IL-1β assay at 10 ng/mL and no cross-reactivity was identified.

Recombinant Human Proteins
- IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-18, IFN-γ, TNF, 41BB-Fc, and GP130

Recombinant Mouse Protein
- IL-1β

Precision

Intra-assay
Twenty four replicates each of three different levels of hIL-1β were tested in one plate. The following results were observed:

<table>
<thead>
<tr>
<th>Number of Replicates (n)</th>
<th>24</th>
<th>24</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Concentration</td>
<td>128.31 pg/mL</td>
<td>67.31 pg/mL</td>
<td>32.55 pg/mL</td>
</tr>
<tr>
<td>SD</td>
<td>3.28</td>
<td>1.67</td>
<td>1.07</td>
</tr>
<tr>
<td>%CV</td>
<td>2.60</td>
<td>2.50</td>
<td>3.30</td>
</tr>
</tbody>
</table>

Inter-assay
Three different levels of hIL-1β were tested in four different plates. The following results were observed:

<table>
<thead>
<tr>
<th>Number of Replicates (n)</th>
<th>32</th>
<th>32</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Concentration</td>
<td>131.98 pg/mL</td>
<td>70.91 pg/mL</td>
<td>33.12 pg/mL</td>
</tr>
<tr>
<td>SD</td>
<td>7.74</td>
<td>3.23</td>
<td>1.03</td>
</tr>
<tr>
<td>%CV</td>
<td>5.86</td>
<td>4.55</td>
<td>3.11</td>
</tr>
</tbody>
</table>

Standardization
This immunoassay is calibrated against recombinant human IL-1β.
The NIBSC/WHO First International Standard 86/680 was evaluated in this kit. The conversion factor for NIBSC material is as follows:

NIBSC (86/680) equivalent value (IU/mL) = 0.010 × BD OptEIA IL-1β value (pg/mL)

\[1.0 \mu g \text{ NIBSC human IL-1β} = 1.395 \mu g \text{ BD OptEIA human IL-1β.}\]
Experimental Results

Serum

Eighteen human serum samples were tested in this assay. The mean value was 4.98 pg/mL, with a range from 0.011 pg/mL to 29.892 pg/mL.

Plasma in Heparin

Nine human plasma samples were tested in this assay. The mean value was 1.61 pg/mL, with a range from 0.142 pg/mL to 6.006 pg/mL.

Plasma in Na Citrate

Seven human plasma samples were tested in this assay. The mean value was 4.721 pg/mL, with a range from 0.080 pg/mL to 20.61 pg/mL.

Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Source</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor Precision</td>
<td>• Inadequate washing / aspiration of wells</td>
<td>• Check function of washing system</td>
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<tr>
<td></td>
<td>• Inadequate mixing of reagents</td>
<td>• Ensure adequate mixing</td>
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<tr>
<td></td>
<td>• Imprecise / inaccurate pipetting</td>
<td>• Check / calibrate pipettes</td>
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<td></td>
<td>• Imprecise sealing of plate</td>
<td>• Ensure complete sealing of plate</td>
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<tr>
<td>Poor Standard Curve</td>
<td>• Improper standard handling / dilution</td>
<td>• Ensure correct preparation of standards</td>
</tr>
<tr>
<td></td>
<td>• Incomplete washing / aspiration of wells</td>
<td>• Check function of washing system</td>
</tr>
<tr>
<td></td>
<td>• Imprecise / inaccurate pipetting</td>
<td>• Check / calibrate pipettes</td>
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<tr>
<td>Low Signal</td>
<td>• Inadequate reagent volumes added to wells</td>
<td>• Check / calibrate pipettes</td>
</tr>
<tr>
<td></td>
<td>• Incorrect incubation times / temperature</td>
<td>• Ensure sufficient incubation times / reagents warmed to room temperature</td>
</tr>
<tr>
<td></td>
<td>• Overly high wash / aspiration pressure from automated plate-washer.</td>
<td>• Utilize manual washing</td>
</tr>
</tbody>
</table>

References


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Plate Templates

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
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For Research Use Only. Not for use in diagnostic or therapeutic procedures.