**Calculation of Results**

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance 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 curve through the standard points.

To determine the IL-1β concentration of the unknowns, find the unknown’s 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 IL-1β concentration by the dilution factor.

**Computer data reduction may also be employed, utilizing log-log regression analysis.**

**Typical Standard Curve**

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

**Specificity**

Cross Reactivity: The following factors were tested in the BD OptEIA™ assay at 10 ng/mL and no cross-reactivity (value ≥ 4 pg/mL) was identified.

Recombinant Human IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-13, IL-15, G-CSF, GM-CSF, IFN-γ, CD23, Lymphotakin, MIP-1α, MIP-1β, MCP-1, MCP-2, NT-3, PDGF-AA, SCF, TNF, IFN-γ, VEGF

Recombinant Mouse IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p70), IL-15, IFN-γ, GM-CSF, MCP-1, TCA3, TNF

Recombinant Rat IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, TNF

Other: Viral IL-10 (1 ng/mL), Rabbit TNF

**Standardization**

This immunoassay is calibrated against recombinant human IL-1β.

The NIBSC/WHO Reference Standard 86/552 (recombinant human IL-1β) was evaluated in this set. The conversion factor for NIBSC material is as follows:

1 μg NIBSC 86/552 human IL-1β = 1.39 μg BD OptEIA™ human IL-1β

**Assay Optimization**

1. BD OptEIA™ Sets allow flexible assay design to fit individual laboratory needs. To design an immunoassay with different sensitivity and dynamic range, the following parameters can be varied: Capture, Detection Antibody titers, Incubation time, Incubation temperature, Assay Diluent formulation, Buffer pH, ionic strength, protein concentration, Type of substrate, Washing technique (i.e., number of wash repetitions and soak times)

2. “Typical Standard Curve” and 20-plate yield were obtained in the BD Biosciences Pharmingen laboratory, using the recommended procedure and manual plate washing.

**Troubleshooting**

**Poor Precision**

Possible Source

- Inadequate washing/ aspiration of wells
- Inadequate mixing of reagents
- Improper/ inaccurate pipetting
- Incomplete sealing of plate

Corrective Action

- Check function of washing system
- Ensure adequate mixing
- Check/ calibrate pipettes
- Ensure complete seal on plate

**Poor Standard Curve**

Possible Source

- Improper standard handling/ dilution standards
- Incomplete washing/ aspiration of wells
- Improper/ inaccurate pipetting
- Improper buffer/ diluent used

Corrective Action

- Ensure correct preparation, storage of standards
- Check function of washing system
- Check/ calibrate pipettes
- Check buffer/ diluent preparation, pH

**Low Absorbances**

Possible Source

- Inadequate reagent volumes added to wells
- Incorrect incubation times/ temperature
- Incorrect antibody titration
- Improper buffer/ diluent used
- Overly high wash/aspiration pressure from automated plate/washer

Corrective Action

- Check/ calibrate pipettes
- Ensure sufficient incubation times/reagents warmed to RT
- Check Capture Ab and Working Detector preparation
- Check buffer/ diluent preparation, pH
- Utilize manual washing

**Limitations of the Procedure**

- Samples that generate absorbance values higher than the standard curve should be diluted with Standard Diluent and re-assayed.
- Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
- BD OptEIA™ Sets are intended for use as an integral unit. Do not mix reagents from different Set batches. Reagents from other manufacturers are not recommended for use in this Set.

**References**


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**Technical Data Sheet**

**Human IL-1β ELISA Set II**

**Materials Provided**

The OptEIA™ Set for human interleukin-1β (IL-1β) contains the components necessary to develop enzyme-linked immunosorbent assays (ELISA) for natural or recombinant human IL-1β in serum, plasma, and cell culture supernatants. Sufficient materials are provided to yield approximately 20 plates of 96-wells if the recommended storage, materials, buffer preparation, and assay procedure are followed as specified in this package.

**Capture Antibody**

Anti-Human IL-1β monoclonal antibody

**Enzyme Reagent**

Streptavidin-horseradish peroxidase conjugate (SAv-HRP)

**Standards**

Recombinant human IL-1β, lyophilized

**Instruction / Analysis Certificate**

(lot-specific)

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**Technical Data Sheet**

**Human IL-1β ELISA Set II**

**Cat. No. 557953**

**Materials Provided**

The OptEIA™ Set for human interleukin-1β (IL-1β) contains the components necessary to develop enzyme-linked immunosorbent assays (ELISA) for natural or recombinant human IL-1β in serum, plasma, and cell culture supernatants. Sufficient materials are provided to yield approximately 20 plates of 96-wells if the recommended storage, materials, buffer preparation, and assay procedure are followed as specified in this package.

**Capture Antibody**

Anti-Human IL-1β monoclonal antibody

**Enzyme Reagent**

Streptavidin-horseradish peroxidase conjugate (SAv-HRP)

**Standards**

Recombinant human IL-1β, lyophilized

**Instruction / Analysis Certificate**

(lot-specific)
1. **Coating Buffer** - 0.1 M Sodium Carbonate, pH 9.5
2. **Assay Diluent** - PBS* with 10% FBS*, pH 7.0. The BD Pharmingen™ Assay Diluent (Cat. No. 555213) is recommended.
3. **Wash Buffer** - PBS* with 0.05% Tween-20. Freshly prepare or use within 3 days of preparation, with 2-8°C storage.
4. **Substrate Solution** - Tetramethylbenzidine (TMB) and Hydrogen Peroxide. The BD Pharmingen™ TMB Substrate Reagent Set (Cat. No. 555214) is recommended.
5. **Stop Solution** - 1 M H2PO4 or 2 N H2SO4

### Additional Materials Required
- 96-well Nunc-Immuno™ polystyrene ELISA flat bottom plates (ThermoFisher Scientific Cat. No. 442404) are recommended.
- Microplate reader capable of measuring absorbance at 450 nm
- Phosphate-Buffered Saline: 80.0 g NaCl, 11.6 g Na2HPO4, 2.0 g KH2PO4, 2.0 g KCl, q.s. to 1 L; pH to 7.0.
- Fetal Bovine Serum: Hyclone Cat. No. SH30088 (heat-inactivated) recommended.
- 8.13 g NaHCO3, 1.59 g Na2CO3; q.s. to 1.0 L; pH to 9.5 with 10N NaOH.

### Storage Information
1. Store unopened reagents at 2-8°C. Do not use reagents after expiration date, or if turbidity is evident.
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 expiration date. See below for reconstituted standard storage information.

### Specimen Collection and Handling
Specimens should be clear, non-hemolyzed and non-lipemic.

### Cell culture supernatants: Remove any particulate material by centrifugation and store immediately or store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

### Serum: Use a serum separator tube and allow samples to clot for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum and assay immediately or store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

### Plasma: Collect plasma using citrate, EDTA, or heparin as anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

### Standards Preparation and Handling
1. **Reconstitution:** After warming lyophilized standard to room temperature, carefully open vial to avoid loss of material. Reconstitute lyophilized standard with 1.0 mL of deionized water to yield a stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Vortex gently to mix.
2. **Storage/handling of reconstituted standard:** After reconstitution, immediately aliquot standard stock in polypropylene vials at 50 µl per vial and freeze at -80°C for up to 6 months. If necessary, store at 2-8°C for up to 8 hours prior to aliquoting/freezing. Do not leave reconstituted standard at room temperature.
3. **Standards Preparation for Assay:**
   a. Prepare a 250 pg/mL standard from the stock standard. Vortex to mix. (See dilution instructions on Instruction/Analysis Certificate.)
   b. Add 300 µL Assay Diluent to 6 tubes. Label as 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL, 7.8 pg/mL, and 3.9 pg/mL.
   c. Perform serial dilutions by adding 300 µL of each standard to the next tube and vortexing between each transfer. Assay Diluent serves as the zero standard (0 pg/mL).

### Recommended Assay Procedure
1. Coat microwells with 100 µL per well of Capture Antibody diluted in Coating Buffer. For recommended antibody coating dilution, see lot-specific Instruction/Analysis Certificate. Seal plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with ≥ 300 µL/well Wash Buffer. After last wash, invert plate and blot on absorbent paper to remove any residual buffer.
3. Block plates with ≥ 200 µL/well Assay Diluent. Incubate at RT for 1 hour.
5. Add 100 µL of Detection Antibody diluted in Assay Diluent to each well. Seal plate and incubate for 1 hour at RT.
6. Aspirate/wash as in step 2, but with 5 total washes.
7. Add 100 µL of Enzyme Reagent diluted in Assay Diluent to each well. Seal plate and incubate for 30 min at RT.
8. Aspirate/wash using 30 second-1 minute soaking steps with 7 total washes.
9. Add 100 µL of Substrate Solution to each well. Incubate plate (without plate sealer) for 30 minutes at room temperature in the dark.
10. Add 50 µL of Stop Solution to each well.
11. Read absorbance at 450 nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract absorbance at 570 nm from absorbance 450 nm.

### Assay Procedure Summary
1. Add 100 µL diluted Capture Ab to each well. Incubate overnight at 4°C.
2. Aspirate and wash 3 times.
3. Block plates: 200 µL Assay Diluent to each well. Incubate 1 hr RT.
4. Aspirate and wash 3 times.
5. Add 100 µL standard or sample to each well. Incubate 2 hr RT.
6. Aspirate and wash 5 times.
7. Add 100 µL Detection Ab to each well. Incubate 1 hr RT.
8. Aspirate and wash 5 times.
9. Add 100 µL diluted SAv-HRP to each well. Incubate 30 min RT.
10. Aspirate and wash 7 times, using 30 sec-1 min soaking.
11. Add 100 µL Substrate Solution to each well. Incubate 30 min RT in dark.
12. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 min with λ correction 570 nm.

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Note: Do not use sodium azide in these preparations. Sodium azide inactivates the horseradish peroxidase enzyme.

The BD OptEIA™ Reagent Set B (Cat. No. 550534) containing Coating Buffer, Assay Diluent, Substrate Reagents A and B, Stop Solution and 20X Wash Buffer Concentrate is recommended.

1. Coating Buffer - 0.1 M Sodium Carbonate, pH 9.5
2. Assay Diluent - PBS* with 10% FBS*, pH 7.0. The BD Pharmingen™ Assay Diluent (Cat. No. 555213) is recommended.
3. Wash Buffer - PBS* with 0.05% Tween-20. Freshly prepare or use within 7 days of preparation, stored at 2-8°C.

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**Hazards Statements**

- **The BD OptEIA™ Reagent Set B** (Cat. No. 550534) containing Coating Buffer, Assay Diluent, Substrate Reagents A and B, Stop Solution and 20X Wash Buffer Concentrate is recommended.
- **Coating Buffer** - 0.1 M Sodium Carbonate, pH 9.5
- **Assay Diluent** - PBS* with 10% FBS*, pH 7.0. The BD Pharmingen™ Assay Diluent (Cat. No. 555213) is recommended.
- **Wash Buffer** - PBS* with 0.05% Tween-20. Freshly prepare or use within 7 days of preparation, stored at 2-8°C.
- **Substrate Solution** - Tetramethylbenzidine (TMB) and Hydrogen Peroxide. The BD Pharmingen™ TMB Substrate Reagent Set (Cat. No. 555214) is recommended.
- **Stop Solution** - 1 M H2PO4 or 2 N H2SO4

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**Recommended Assay Procedure**

1. Coat microwells with 100 µL per well of Capture Antibody diluted in Coating Buffer. For recommended antibody coating dilution, see lot-specific Instruction/Analysis Certificate. Seal plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with ≥ 300 µL/well Wash Buffer. After last wash, invert plate and blot on absorbent paper to remove any residual buffer.
3. Block plates with ≥ 200 µL/well Assay Diluent. Incubate at RT for 1 hour.
5. Prepare standard and sample dilutions in Assay Diluent. See “Standards Preparation and Handling”.
6. Pipette 100 µL of each standard, sample, and control into appropriate wells. Seal plate and incubate for 2 hours at RT.
7. Aspirate/wash as in step 2, but with 5 total washes.
8. Add 100 µL of Detection Antibody diluted in Assay Diluent to each well. Seal plate and incubate for 1 hour at RT.
9. Aspirate/wash as in step 2, but with 5 total washes.
10. Add 100 µL of Enzyme Reagent diluted in Assay Diluent to each well. Seal plate and incubate for 30 min at RT.
11. Aspirate/wash using 30 second-1 minute soaking steps with 7 total washes.
12. Add 100 µL of Substrate Solution to each well. Incubate plate (without plate sealer) for 30 minutes at room temperature in the dark.
13. Add 50 µL of Stop Solution to each well.
14. Read absorbance at 450 nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract absorbance at 570 nm from absorbance 450 nm.

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**Assay Procedure Summary**

1. Add 100 µL diluted Capture Ab to each well. Incubate overnight at 4°C.
2. Aspirate and wash 3 times.
3. Block plates: 200 µL Assay Diluent to each well. Incubate 1 hr RT.
4. Aspirate and wash 3 times.
5. Add 100 µL standard or sample to each well. Incubate 2 hr RT.
6. Aspirate and wash 5 times.
7. Add 100 µL Detection Ab to each well. Incubate 1 hr RT.
8. Aspirate and wash 5 times.
9. Add 100 µL diluted SAv-HRP to each well. Incubate 30 min RT.
10. Aspirate and wash 7 times, using 30 sec-1 min soaking.
11. Add 100 µL Substrate Solution to each well. Incubate 30 min RT in dark.
12. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 min with λ correction 570 nm.