**Mouse IL-1β**

**ELISA MAX™ Standard Sets**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>432601</td>
<td>5 plates</td>
<td>Mouse IL-1β ELISA MAX™ Capture Antibody (200X)</td>
</tr>
<tr>
<td>432602</td>
<td>10 plates</td>
<td>Mouse IL-1β ELISA MAX™ Detection Antibody (200X)</td>
</tr>
<tr>
<td>432603</td>
<td>20 plates</td>
<td>Mouse IL-1β Standard</td>
</tr>
<tr>
<td>432604</td>
<td>1000X</td>
<td>Avidin-HRP</td>
</tr>
</tbody>
</table>

BioLegend's ELISA MAX™ Standard Sets contain the capture and detection antibodies, recombinant protein standard, and Avidin-HRP required for the accurate quantification of natural and recombinant mouse IL-1β. These sets are cost-effective and designed for experienced ELISA users. It is highly recommended that the instruction sheet be read in its entirety before using this product. Use the recommended assay protocol, microwell plates, buffers, diluent, and substrate solution to obtain desired assay results. Do not use this set beyond the expiration date.

**Materials to be Provided by the End-User**

- Microwell plates: BioLegend Cat. No. 423501 is recommended.
- Plate Seals: BioLegend Cat. No. 423601 is recommended.
- Phosphate-Buffered Saline (PBS): 8.0 g NaCl, 1.16 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, add deionized water to 1.0 L, pH to 7.4.
- Coating Buffer: 8.4 g NaHCO₃, 3.56 g Na₂CO₃, add deionized water to 1.0 L, pH to 9.5 (BioLegend Cat. No. 421701 is recommended).
- Assay Diluent: 10% Fetal Bovine Serum or 1% BSA in PBS (BioLegend Cat. No. 421203 is recommended).
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20.
- TMB Substrate Solution: BioLegend Cat. No. 421101 is recommended.
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2 N H₂SO₄.
- A microplate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes to measure volumes ranging from 2 μL to 1 mL.
- Deionized (DI) water.
- Wash bottle or automated microplate washer.
- Log-Log graph paper or software for data analysis.
- Tubes to prepare standard dilutions.
- Timer.
- Absorbent paper.

**Reagent and Sample Preparation**

Do not mix reagents from different sets or lots. Avidin-HRP, Mouse IL-1β Standard, and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

1. Dilute the pre-titrated Capture Antibody 1:200 in Coating Buffer. For one plate, dilute 60 μL Capture Antibody in 11.94 mL Coating Buffer.
2. Reconstitute the lyophilized standard with 0.2 mL of Assay Diluent, re-cap vial, and mix well. Allow the reconstituted standard to sit for 15 minutes at room temperature, then invert/vortex to mix well.
3. Prior to use, prepare 1,000 μL of the top standard at a concentration of 2,000 pg/mL from stock solution in Assay Diluent (refer to Lot-Specific Certificate of Analysis/ELISA MAX™ Standard Set Protocol). Perform six two-fold serial dilutions of the 2,000 pg/mL top standard with Assay Diluent in separate tubes. After diluting, the mouse IL-1β standard concentrations are 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, and 31.3 pg/mL, respectively. Assay Diluent serves as the zero standard (0 pg/mL).
4. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in Assay Diluent. For one plate, dilute 60 μL Detection Antibody in 11.94 mL Assay Diluent.
5. Dilute Avidin-HRP 1:1000 in Assay Diluent. For one plate, dilute 12 μL Avidin-HRP in 11.99 mL Assay Diluent.
6. Prepare all other reagents required for the assay including TMB Substrate Solution. Refer to reagent description in the section “Materials to be Provided by the End-User”.
7. Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilutions are necessary, samples should be diluted in the corresponding cell culture medium. For other sample types, such as serum and plasma, optimization of reagent concentrations and assay conditions may be required.

**Materials Provided**

1. Mouse IL-1β ELISA MAX™ Capture Antibody (200X)
2. Mouse IL-1β ELISA MAX™ Detection Antibody (200X)
3. Mouse IL-1β Standard
4. Avidin-HRP (1000X)
5. Lot-Specific Certificate of Analysis/ELISA MAX™ Standard Set Protocol

**Introduction**

IL-1 refers to two proteins, IL-1α and IL-1β which are the products of distinct genes, but which are recognized by the same cell surface receptors. IL-1 is a potent immuno-modulator which mediates a variety of biological responses, including the activation of B and T cells.

**Principle of the Test**

BioLegend’s ELISA MAX™ Standard Set is a sandwich Enzyme-Linked Immunosorbsent Assay (ELISA). A mouse IL-1β specific Armenian hamster monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL-1β binds to the immobilized capture antibody. Next, a biotinylated goat polyclonal anti-mouse IL-1β detection antibody is added, producing an antibody-antigen-antibody “sandwich”. Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of IL-1β present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.
Assay Procedure Summary

**Day 1**
Add 100 μL diluted Capture Antibody solution to each well, incubate overnight at between 2°C and 8°C.

**Day 2**
1. Wash plate 4 times
2. Add 200 μL Assay Diluent to block, incubate at room temperature for 1 hour with shaking
3. Wash plate 4 times
4. Add diluted standards and samples to the appropriate wells, incubate at room temperature for 2 hours with shaking
5. Wash plate 4 times
6. Add 100 μL diluted Detection Antibody solution to each well, incubate at room temperature for 1 hour with shaking
7. Wash plate 4 times
8. Add 100 μL diluted Avidin-HRP solution to each well, incubate at room temperature for 30 minutes with shaking
9. Wash plate 5 times, soaking for 30 seconds to 1 minute per wash
10. Add 100 μL of TMB Substrate Solution to each well, incubate in the dark for 15-30 minutes or until the desired color develops*
11. Add 100 μL Stop Solution to each well
12. Read absorbance at 450 nm and 570 nm

*Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.

Calculation of Results

Plot the standard curve on log-log axis graph paper with cytokine concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations in the samples, find the absorbance value of the unknown 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 corresponding cytokine concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

**Standard Curve:** This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.

Performance Characteristics

**Specificity:** No cross reactivity was observed when this kit was used to analyze multiple human, mouse and rat recombinant proteins.

Troubleshooting

High Background:
- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:
- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contained sodium azide.

Low or poor signal for the standard curve:
- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with inappropriate temperature, timing or agitation.

Signal too high, standard curves saturated:
- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:
- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:
- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

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For other technical resources, please visit:
www.biolegend.com/support or email: techserv@biolegend.com

For more information about BioLegend ELISA MAX™ Sets and LEGEND MAX™ ELISA Kits with precoated plates, visit www.biolegend.com.