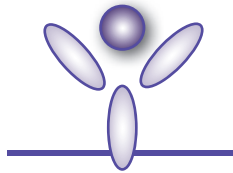# BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human IL-1β

Cat. No. 437007

ELISA Kit for Accurate Quantitation of Human IL-1β from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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LEGEND MAX™ Human IL-1β ELISA Kit

Introduction:

Human IL-1β (Interleukin-1β, Catabolin, Hematopoietin-1 (H1), IFN-β-inducing factor, interleukin-β, Osteoclast activating factor (OAF)). IL-1 refers to two proteins (IL-1α and IL-1β), which are the products of distinct genes that are recognized by the same cell surface receptors. IL-1β is a potent immuno-modulator that mediates a wide range of immune and inflammatory responses, including the activation of B and T cells. IL-1β is expressed in several cell types, including monocytes, tissue macrophages, Langerhan cells, dendritic cells, T lymphocytes, B lymphocytes and natural killer cells. IL-1β is upregulated by TNF-α, IFN-α, IFN-β, IFN-γ, bacterial endotoxins, viruses, mitogens, and antigens. IL-1β is downregulated by IL-6, lipoproteins, lipids, and α2-macroglobulin. IL-1β plays a role in many physiological and pathological conditions, including rheumatoid arthritis and cancer.

The BioLegend LEGEND MAX™ Human IL-1β ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal mouse anti-human IL-1β capture antibody. The detection antibody is a biotinylated polyclonal goat anti-human IL-1β antibody. This kit is specifically designed for the accurate quantitation of human IL-1β from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-human IL-1β Pre-coated 96-well Strip Microplate	1 plate		79379
Human IL-1β Detection Antibody	1 bottle	12 mL	79380
Human IL-1β Standard	1 vial	lyophilized	78164
Streptavidin-Polymer HRP	1 bottle	12 mL	750002513
Assay Buffer D	1 bottle	25 mL	79383
Matrix E (for serum and plasma samples only)	1 vial	lyophilized	79378
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included des-iccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Matrix E	
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Streptavidin-Polymer HRP	
Assay Buffer D	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).

2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.

3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*

5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.

2. If serum or plasma samples will be assayed, reconstitute the lyophilized Matrix E by dispensing 2 mL of deionized water into the vial and allow the reconstituted Matrix E to sit at room temperature for 15 minutes, then vortex to mix completely.

3. Reconstitute the lyophilized Human IL-1β Standard by adding the volume of Assay Buffer D to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis). Allow the

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reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.

4. In general, samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer D for diluting cell culture supernatant samples and Matrix E for diluting plasma and serum samples.

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 125 pg/mL top standard by diluting the appropriate amount of the standard stock solution in Assay Buffer D (refer to the LEGEND MAX Kit Lot-Specific Certificate of Analysis). Perform six two-fold serial dilutions of the 125 pg/mL top standard in separate tubes using Assay Buffer D as the diluent. Thus, the human IL-1β standard concentrations in the tubes are 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, 7.8 pg/mL, 3.9 pg/mL and 2 pg/mL respectively. Assay Buffer D serves as the zero standard (0 pg/mL).
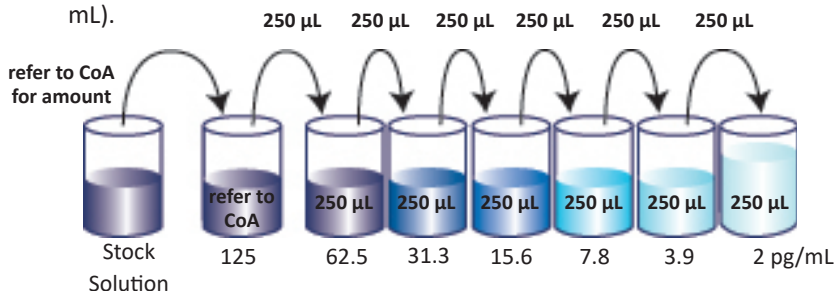
4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. **For measuring samples of cell culture supernatant:**
    a) Add 50 µL of Assay Buffer D to each well that will contain either standard dilutions or samples.
    b) Add 50 µL of standard dilutions or samples to the appropriate wells.
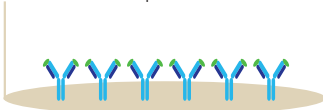
For measuring serum or plasma samples:
a) Add 50 μL of Matrix E to each well that will contain the standard dilutions. Add 50 μL of Assay Buffer D to each well that will contain samples.
b) Add 50 μL of standard dilutions to the wells containing Matrix E. Add 50 μL of diluted serum or plasma samples to the wells containing Assay Buffer D.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

8. Add 100 μL of Human IL-1β Detection Antibody to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

10. Add 100 μL of Streptavidin-Polymer HRP to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

12. Add 100 μL of Substrate Solution F to each well and incubate for 20 minutes* in the dark. Wells containing human IL-1β should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 μL of Stop Solution to each well. The solution color should change from blue to yellow.

14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

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

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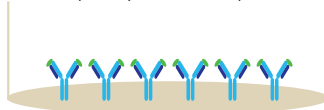
Assay Procedure Summary

1.

For measuring cell culture supernatant:

Wash 4 times.
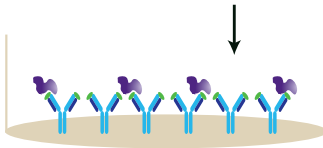Add 50 µL Assay Buffer D to standard and sample wells.
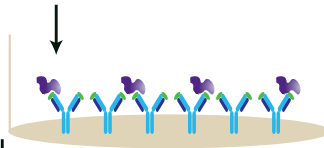
For measuring serum or plasma:

Wash 4 times.
Add 50 µL Matrix E to standard wells.
Add 50 µL Assay Buffer D to sample wells.

2. Add 50 µL diluted standards to standard wells.
Add 50 µL samples to sample wells.
Incubate 2 hr, RT on plate shaker.

Add 50 µL diluted standards to standard wells
Add 50 µL of samples to sample wells
Incubate 2 hr, RT on plate shaker.

3. Wash 4 times.
Add 100 µL Detection Antibody.
Incubate 1 hr, RT on plate shaker.
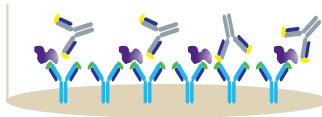
4. Wash 4 times.
Add 100 µL Streptavidin-Polymer HRP.
Incubate 30 min, RT on plate shaker.
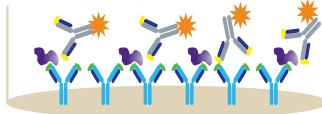
5. Wash 5 times.
Add 100 µL Substrate Solution F.
Incubate 20 min*, RT in the dark.

6. Add 100 µL Stop Solution.

7. Read absorbance at 450 nm and 570 nm.

Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper wit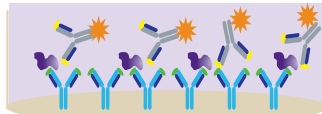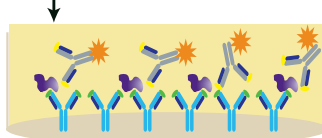h cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

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

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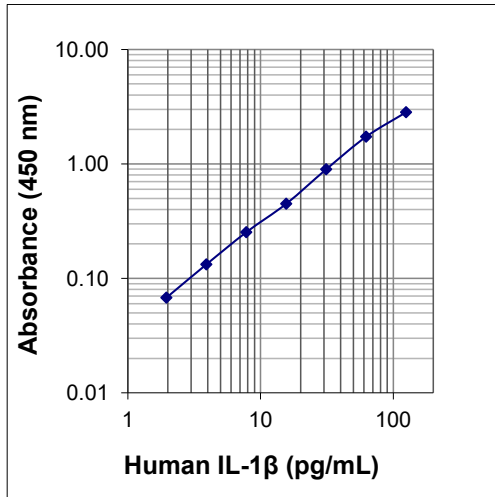
Performance Characteristics:

Specificity: No cross reactivity was observed when this kit was used to analyze 15 human recombinant cytokines/chemokines and mouse IL-1β at up to 50 ng/mL.

Sensitivity: The minimum detectable concentration of IL-1β is 0.5 pg/mL.

Recovery: Recombinant IL-1β was spiked into 4 human serum samples at concentrations of 125 pg/mL, 31.3 pg/mL, and 7.8 pg/mL then analyzed with the LEGEND MAX™ Human IL-1β ELISA kit. On average, 103.1 % of the cytokine was recovered from the serum samples.

Linearity: Four human serum samples spiked with high concentrations of IL-1β were diluted with the appropriate matrix to produce samples with concentrations within the dynamic range and then assayed. On average, 101 % of the expected cytokine was detected from the serum samples

Intra-Assay Precision: Sixteen replicates of each of two samples containing different IL-1β concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	51.8	15.9
Standard Deviation	6.8	0.96
% CV	11.6	6.0

Inter-Assay Precision: Two samples containing different concentrations of IL-1β were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	56.5	16.3
Standard Deviation	7.4	1.2
% CV	13.1	7.2

Biological Samples:

Serum - Twenty human serum samples from randomly selected apparently healthy donors were assayed for basal levels of human IL-1β. Two samples measured at 1.9 pg/mL and 4.25 pg/ml, and eighteen samples measured less than the lowest IL-1β standard curve point, 2 pg/mL.

Cell Culture Supernates - Freshly isolated human PBMC at a concentration of 1×10^6 cells/mL were stimulated with 100 ng/mL LPS, or 50 ng/mL PMA plus 1 µg/mL ionomycin at 37°C for 3 days. The cell culture supernatants were collected and assayed for levels of natural human IL-1β. The resulting human IL-1β concentration averaged 2,704 pg/mL in LPS-stimulated samples, 2,185 pg/mL in PMA and ionomycin double-stimulated samples and 3.4 pg/mL in unstimulated samples.

LEGEND MAX™ Human IL-1β ELISA Kit

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers.
		Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

LEGEND MAX™ Human IL-1β ELISA Kit

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Notes

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LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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