BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plate

Human IL-1α

Cat. No. 445807

ELISA Kit for Accurate Quantitation of Human IL-1α from Cell Culture Supernatant, Serum, and Plasma

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.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.

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Introduction:

Interleukin-1 (IL-1) refers to two proteins, IL-1α and IL-1β, which are from different gene products. IL-1α and IL-1β share about 25% amino acid sequence identity. They are initially synthesized as 31 kD precursors, and then cleaved by proteases into mature proteins with molecular mass approximately of 17 kD.

IL-1 binds to both forms of the cell surface type I (IL-1RI) and type II (IL-1RII) IL-1 receptors. IL-1RI is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes while IL-1RII is found on B-cell lineages, neutrophils, and bone marrow cells. Although both IL-1α and IL-1β are recognized by IL-1RI and IL-1RII receptors, IL-1α binds better to the type I receptor than IL-1β and vice versa.

IL-1 has different biological properties such as immunologic, pro-inflammatory, and protective effects. An increase of IL-1 by a variety of cells is a good indicator of infection by microbial toxins and inflammatory processes. It also affects B cell activation, immunocompetent cells and other immunologic cells. Studies show that the production of IL-1 has been detected in human joint fluids from patients with rheumatoid arthritis, osteoarthritis, traumatic arthritis, and psoriatic arthritis. In addition, IL-1α shows elevated levels in cancer such as colon cancer and cervical cancer.

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

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Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-human IL-1α Precoated 96-well Strip Microplate	1 plate		78284
Human IL-1α Detection Antibody	1 bottle	12 mL	77664
Human IL-1α Standard	1 vial	lyophilized	78155
Avidin-HRP	1 bottle	12 mL	77897
Assay Buffer D	1 bottle	25 mL	79383
Matrix G (for EDTA plasma samples only)	1 vial	lyophilized	77655
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

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 Strips	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 desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution or Matrix G can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Matrix G	
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP	
Assay Buffer D*	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

***Note: Precipitate may form in Assay Buffer D. If present, heat buffer to 37°C and vortex. Any remaining precipitate will have no effect on assay performance.**

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 and is corrosive. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

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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 should 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 20 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 20 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:

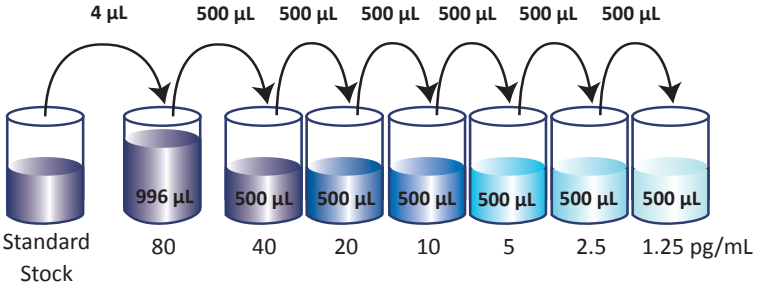
Note: All reagents should be diluted immediately prior to use.

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 EDTA plasma samples will be assayed, reconstitute the lyophilized Matrix G by dispensing 2 mL of deionized water into the vial and allow the reconstituted Matrix G to sit at room temperature for 15 minutes, then vortex to mix completely.
3. Reconstitute the lyophilized Human IL-1α Standard by adding the volume of Assay Buffer D to make a 20 ng/mL standard stock solution (Refer to LEGEND MAX™ Kit Lot-Specific Certificate of Analysis/LEGEND MAX™ Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 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 serum, plasma (Heparin and Citrate), cell culture supernatant samples and Matrix G for diluting EDTA plasma 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 1,000 µL of the 80 pg/mL top standard by diluting 4 µL of the standard stock in 996 µL of Assay Buffer D. Perform six two-fold serial dilutions of the 80 pg/mL top standard in separate tubes using Assay Buffer D as the diluent. Thus, the Human IL-1α standard concentrations in the tubes are 80 pg/mL, 40 pg/mL, 20 pg/mL, 10 pg/mL, 5 pg/mL, 2.5 pg/mL, and 1.25 pg/mL, respectively. Assay Buffer D serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean, absorbent paper. All subsequent washes should be performed similarly.
5. For measuring samples of serum, plasma (heparin and citrate) and cell culture supernatant:
   a) Add 50 µL of Assay Buffer D to each well that will contain either standard dilutions or sample.
   b) Add 50 µL of standard dilutions or samples to the appropriate wells.

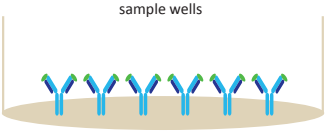
   For measuring EDTA plasma samples:
   a) Add 50 µL of Matrix G to each well that will contain the standard dilutions. Add 50 µL of Assay Buffer D to each well that will contain samples.
   b) Add 50 µL of standard dilutions to the wells containing Matrix G. Add 50 µL of EDTA plasma samples to the wells 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-1α Detection Antibody solution to each well, seal and incubate the plate 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 Avidin-HRP solution 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 at room temperature for 15 minutes in the dark. Wells containing human IL-1α 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 immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

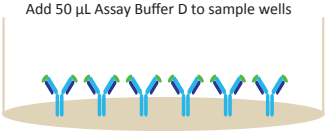
1. **For measuring serum, plasma (Heparin and Citrate), and cell cuture supernant:**

Wash 4 times
Add 50 µL Assay Buffer D to standard and sample wells

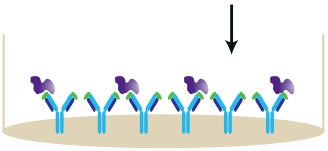


For measuring EDTA plasma:

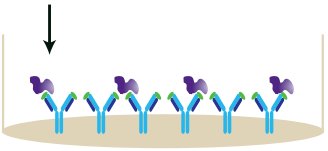
Wash 4 times
Add 50 µL Matrix G to standard wells
Add 50 µL Assay Buffer D to sample wells



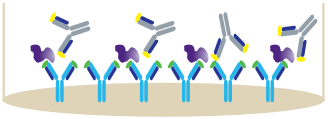
2. Add 50 µL diluted standards to standard wells
Add 50 µL samples to sample wells
Incubate 2 hr, RT, shaking



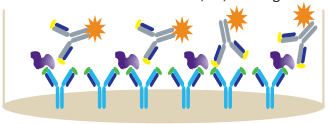
Add 50 µL diluted standards to standard wells
Add 50 µL diluted samples to sample wells
Incubate 2 hr, RT, shaking



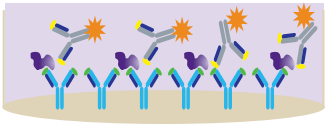
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



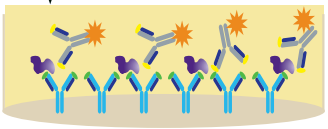
4. Wash 4 times
Add 100 µL Avidin-HRP solution
Incubate 30 min, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 15 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

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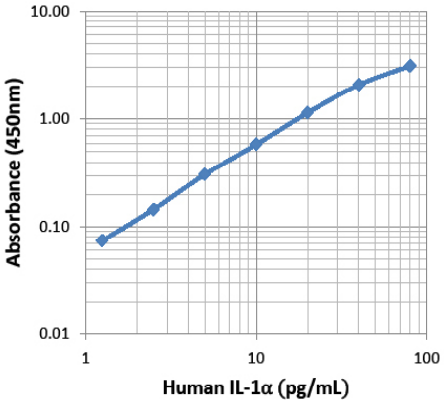
Calculation of Results:

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 with protein concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown protein 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 protein 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.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze the following recombinant proteins, each at 50 ng/mL.

Human Proteins	EGF, EPO, IFN-β1, IL-1β, IL-1RA, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-17A, IL-17AF, IL-21, IL-22, IL-23, IL-27, TGF-α, TGF-β1, TGF-β2, VEGF-165, VEFG-121.
Mouse Proteins	IL-1α, IL-1β, IL-3

Sensitivity: The minimum detectable concentration of human IL-1α is 0.25 pg/mL ± 0.08 pg/mL (n=8).

Recovery: Recombinant human IL-1α at concentrations of 50, 12.5, and 3.1 pg/mL, was spiked into the following human samples, then analyzed with the LEGEND MAX™ Human IL-1α ELISA Kit.

Sample Type	N	% Recovery
Serum	4	98
EDTA Plasma	4	91
Heparin Plasma	4	88
Citrate Plasma	4	88

Linearity: Recombinant human IL-1α was spiked into human serum and plasma samples to produce samples within the dynamic range, and then assayed with the kit to determine the dilutional linearity up to 8-fold dilutions. Alternatively, stimulated human PBMC containing high levels of IL-1α was diluted 2-fold with Assay Buffer D and assayed up to 128-fold dilutions.

Sample Type	N	% Linearity
Serum	4	109
EDTA Plasma	4	109
Heparin Plasma	4	100
Citrate Plasma	4	89
Stimulated PBMC	1	107

Intra-Assay Precision: Two serum samples was spiked with different recombinant human IL-1α concentrations, were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	22.2	14.2
Standard Deviation	1.3	0.5
% CV	5.9	3.5

Inter-Assay Precision: Two serum samples was spiked with different concentrations of recombinant human IL-1α, were assayed in four independent assays by different operators.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	22.4	14.1
Standard Deviation	0.8	0.7
% CV	5.8	3.0

Biological Samples:
Normal Ranges: Twenty four serum and plasma (Heparin, EDTA, and Citrate) samples from different healthy donor were tested for endogenous levels of IL-1α. The levels were measured less than the lowest standard, 1.25 pg/mL.

Cell Culture Supernatant - Human PBMC (2 x 10^6 cells/mL) was stimulated with 1 μg/mL LPS for up to 2 days. Cell culture supernatants were collected and analyzed for natural levels of human IL-1α. The concentration of human IL-1α was 240 pg/mL in LPS-stimulated samples and was not detectable in unstimulated samples.

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.

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.

ELISA Plate Template

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

Notes

Notes

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Notes

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