# Human IL-1β ELISA Kit

EZHIL1B

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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## 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, but which are recognized by the same cell surface receptors. IL-1β is a potent immunomodulator which 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-1 β is upregulated by TNF-α, IFN-α, IFN-β, IFN-γ, bacterial endotoxins, viruses, mitogens, and antigens; while IL-1β is downregulated by IL-6, lipoproteins, lipids, and α2-macroglobulin. IL-1 β plays a role in many physiological and pathological conditions including rheumatoid arthritis and cancer.
The EMD Millipore ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with 96-well strip plates that are pre-coated with a capture antibody.

The EMD Millipore ELISA kits with pre-coated plates are specifically designed for the accurate quantification of analytes from cell culture supernatant, serum, plasma or other body fluids. All kits are analytically validated and provide ready-to-use reagents.

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## Materials Provided

| Store at 2℃ to 8℃ | | |
|---|---|---|
| **Component** | **Item No.** | **Volume** |
| Anti-Human IL-1β pre-coated 96-well plate | CS211391 | 1 plate |
| Human IL-1β Detection Antibody | CS211278 | 1 bottle (12 mL) |
| Human IL-1β Standard | CS211393 | 1 vial (lyophilized) |
| Avidin-HRP D | CS210086 | 1 bottle (12 mL) |
| Assay Buffer D | CS211407 | 1 bottle (25 mL) |
| Matrix E (for serum and plasma samples only) | CS211406 | 1 vial (lyophilized) |
| Wash Buffer (20X) | CS210053 | 1 bottle (50 mL) |
| Substrate Solution F | CS210054 | 1 bottle (12 mL) |
| Stop Solution | CS213422 | 1 bottle (12 mL) |
| Plate Sealers | CS210056 | 4 sheets |

## Materials Required But Not Supplied

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

## Warnings and Precautions

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and plasma in accordance with NCCLS regulations.
- Before disposing the plate at end.

## Storage and Stability

Store at 2℃-8ºC; performance guaranteed for 4 mont hs from date of receipt when reagents are stored properly.

## Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to dtermine an optimal dilution factor to ensure accurate quantification.
Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at -70℃. 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℃. Avoid repeated freeze/thaw cycles.
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70℃. Avoid repeated freeze/thaw cycles.

## Reagent and Sample Preparation

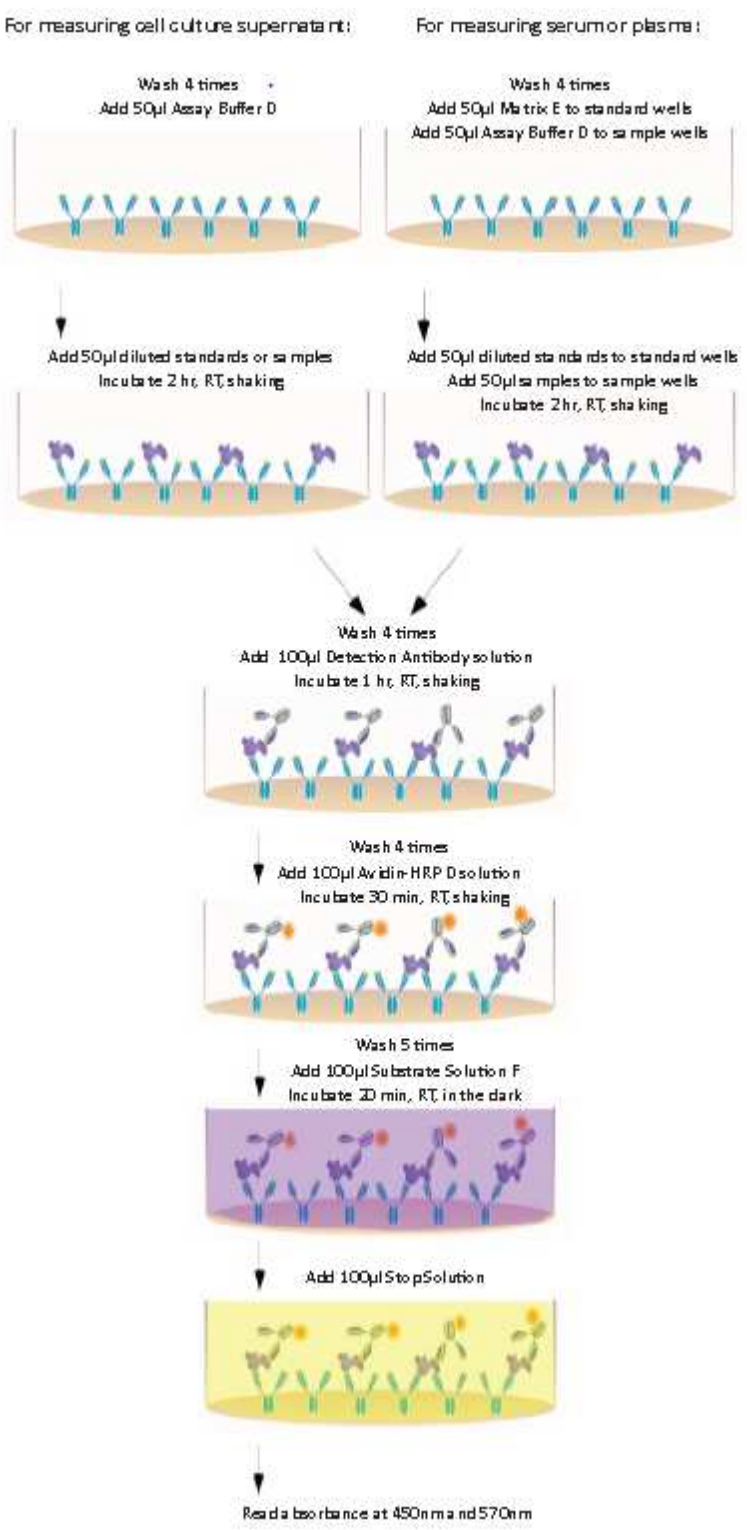
1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.
2. 2. Reconstitute the lyophilized Human IL-1β Standard by adding the volume of Assay Buffer D described on the vial label to make the 20 ng/mL standard stock solution, mix gently. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, vortex again to mix completely.
3. 3. 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.
4. 4. In general, samples are analyzed without dilutions. If dilutions are necessary, samples should be diluted:
5. For measuring cell culture supernatant samples: Samples should be diluted with Assay Buffer D.
6. For measuring serum or plasma samples: Samples should be diluted with Matrix E.

## Protocol

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

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. Prepare 500 µL of the 125 pg/mL top standard by diluting 3.13 µL of the standard stock solution in 496.9 µL Assay Buffer D. Perform six two-fold serial dilutions of the 125 pg/mL top standard in separate tubes. 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).
3. Wash plate 4 times with at least 300 µl 1X Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.
4. **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/well of standard dilutions or samples to the appropriate wells.
5. **For measuring serum or plasma samples:**
    a. Add 50 µL/well of Matrix E to each well that will contain the standard dilutions and 50 µL/well of Assay Buffer D to each well that contains samples.
    b. Add 50 µL/well of the prepared standard dilutions to the standard wells and 50 µL/well of serum or plasma samples to the sample wells.
6. Seal the plate with a Plate Sealer provided in the kit and then incubate at room temperature for 2 hours with shaking at 200 rpm on a plate shaker.
7. Discard the plate contents in a sink, then wash the plate 4 times with 1X Wash Buffer as in step 3.
8. Add 100 µL of the Human IL-1β Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour with shaking.
9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 3.
10. Add 100 µL of Avidin-HRP D solution to each well, seal the plate and incubate at room temperature for 30 minutes with shaking.
11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 3. 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 with higher concentrations of human IL-1β should turn a blue color. 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 blue color should change to yellow color.
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.

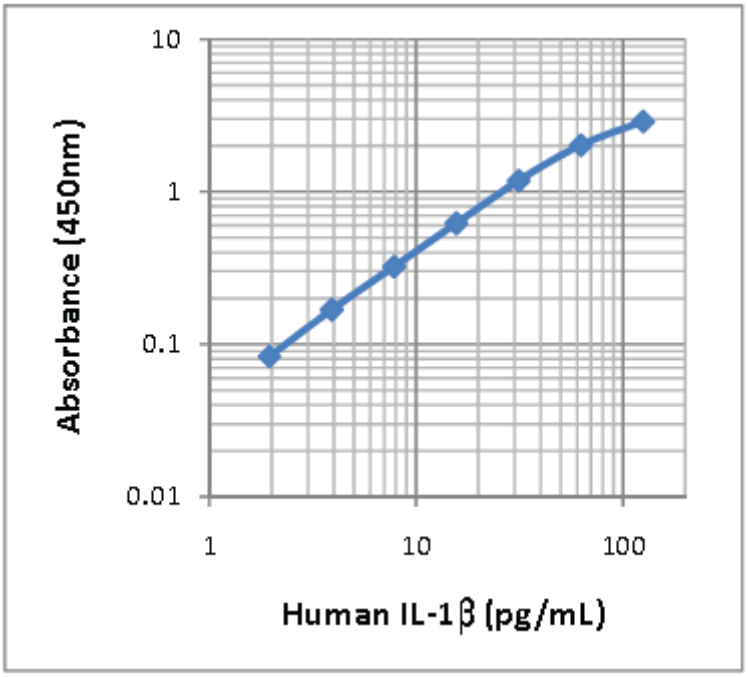
# Assay Procedure Summary



## Data Analysis

The data can be best calculated with omputer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If 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 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 for demonstration purposes only. A standard curve must be run with each assay.




## Product Performance

*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 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-1b. Two samples measured at 1.9 pg/mL and 4.25 pg/ml, and eighteen samples measured less than the lowest IL-1b standard curve point, 2 pg/mL.
- *Cell Culture Supernates* - Freshly isolated human PBMC at a concentration of 1 x $10^6$ cells/mL were stimulated with 100 ng/mL LPS, or 50 ng/mL PMA plus 1 µg/ mL ionomycin at 37℃ 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.

## Trouble Shooting Guide:

| Problem | Probable Cause | Solutions |
|---|---|---|
| High Background | Background wells were contaminated | Avoid cross-well contamination by using the provided plate sealers.<br>Use multichannel pipettes and change tips between pipetting samples and reagents. |
| | Insufficient washes | Increase number of washes.<br>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 ($NaN_3$) | 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℃. Avoid repeated freeze-thaw cycles. |
| | Reagents added to wells with incorrect concentrations | Check for pipetting errors and the correct reagent volume. |

## Trouble Shooting Guide:

| Problem | Probable Cause | Solutions |
|---|---|---|
| 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.<br>Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate. |

# Microtiter Plate Map

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

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