# EZElisa™ Mouse Interleukin 1 Beta (IL1b) ELISA Kit

Cat #: A-QEK10285

Size: 96wells

Storage: All reagents should be stored as indicated on the component label.

## Product information

### Introduction

Mouse Interleukin 1β (IL-1β), also called lymphocyte activating factor (LAF), endogenous pyrogen (EP), leucocyte endogenous mediator (LEM), mononuclear cell factor (MCF), is a ~17 kDa factor produced by a wide variety of cells, including macrophages, dendritic cells, T and B cells. IL-1β is mostly cell associated with 23% amino acid homology with IL-1α. The immune regulatory role of IL-1β is exerted on a wide range of cells including lymphocytes, epithelial cells and fibroblasts. In vivo, it induces hypotension, fever, and acute phase response.

### Intended Use

EZElisa™ Mouse Interleukin 1 Beta (IL1b) ELISA Kit is specifically designed for the accurate quantitation of Mouse Interleukin 1β (IL-1β) from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

### Materials Provided

1. Microtiter Coated Plate (12X8 wells) – 1 plate
2. Recombinant Mouse Interleukin 1β (IL-1β) Standard lyophilized (110 ng/ml) – 1 vial
3. Mouse Interleukin 1β (IL-1β) Biotin Conjugated Detection Antibody lyophilized (15 ug/ml) – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (20X) Wash Buffer – 25ml
6. (1X) Assay Diluent – 50ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml

9. Instruction Manual

### Materials to be provided by the End-User

1. Microplate Reader able to measure absorbance at 450nm.

2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.

3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.

5. Graph paper or software for data analysis.

6. Tubes to prepare standard/sample dilutions.

7. Timer.

8. Absorbent paper.

### Storage Information

1. Store main kit components at 2-8°C.

2. Store recombinant Standard and Detection at 2-8°C. Upon reconstitution, aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.

3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

### Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

## Procedure

### Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate

quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature < -20°C. Avoid repeated freeze/thaw cycles.

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

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

## Reagent Preparation

Please refer to lot specific instructions for preparation of the reagents.

## Assay Procedure

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

2. Standards Preparation: Reconstitute the lyophilized vial with 40 ul of Assay Diluent (1X) to generate a 110 ng/ml standard concentration. Perform dilutions by using main stock solution as per the below table. Thus the Mouse Interleukin 1β (IL-1β) Standards concentration are 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml and 15.63pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

| Standard Concentration | Standard No | Dilution Particulars |
|---|---|---|
| 110 ng/ml (lyophilized) | Standard Main stock | Original Standard (lyophilized) + 40 ul Assay diluent (1X) |
| 1000 pg/ml | Standard No.7 | 9.09ul Main stock + 990.91 ul Assay diluent (1X) |
| 500 pg/ml | Standard No.6 | 500 ul Standard No.7 + 500 ul Assay diluent (1X) |
| 250 pg/ml | Standard No.5 | 500 ul Standard No.6 + 500 ul Assay diluent (1X) |
| 125 pg/ml | Standard No.4 | 500 ul Standard No.5 + 500 ul Assay diluent (1X) |
| 62.5 pg/ml | Standard No.3 | 500 ul Standard No.4 + 500 ul Assay diluent (1X) |
| 31.3 pg/ml | Standard No.2 | 500 ul Standard No.3 + 500 ul Assay diluent (1X) |
| 15.63 pg/ml | Standard No.1 | 500 ul Standard No.2 + 500 ul Assay diluent (1X) |

3. Add 100µl/well of Standards and Samples to the plate, Seal plate and incubate for 2 hours at Room Temperature (18-25°C).

4. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

5. Add 100µl of diluted Detection Antibody solution to each well, seal plate and incubate for 1 hour at Room Temperature (18-25°C).

6. Wash plate 4 times with Wash Buffer (1X) as in step 4.

7. Add 100µl of diluted Streptavidin-HRP solution to each well, seal plate and incubate for 30 minutes at Room Temperature (18-25°C).

8. Wash plate 4 times with Wash Buffer (1X) as in step 4.

9. Add 100µl of TMB Substrate solution and incubate in the dark for 15 minutes at Room Temperature (18-25°C). Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

10. Stop reaction by adding 100µl of Stop Solution to each well. Positive wells should turn from blue to yellow.

11. Read absorbance at 450 nm within 30 minutes of stopping reaction.

## Calculation of Results

Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns 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 cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software may be preferred. Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

### Performance Characteristics

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

**Sensitivity:**

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to 15 pg/ml.

**Specificity:**

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Mouse Interleukin 1β (IL-1β).

**Assay Range:**

15.63 pg/ml to 1000 pg/ml

**Precision:**

Intra-Assay: CV<10%

Inter-Assay: CV<12%

**Linearity:**

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Mouse Interleukin 1β (IL-1β) and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

| Sample | 1:2 | 1:4 | 1:8 |
|---|---|---|---|
| Serum(n=5) | 84-107% | 87-108% | **82-112%** |
| EDTA plasma(n=5) | 83-102% | 83-115% | **83-118%** |
| Heparin plasma(n=5) | 83-99% | 80-95% | **82-93%** |

## Safety Precautions

• This kit is For Research Use only. Follow the working instructions carefully.

• The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.

• Do not use or mix reagents from different lots.

• Do not use reagents from other manufacturers.

• Avoid time shift during pipetting of reagents.

• All reagents should be kept in the original shipping container.

• Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.

• Source materials maybe derived from Mouse body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

• Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material

- Always use protective gloves

- Never pipette material by mouth

- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

• In any case GLP should be applied with all general and individual regulations to the use of this kit.

## SYMBOLS KEY

| | |
|---|---|
| MTP | Microtiter Plate (12X8 wells) |
| STD | Mouse Interleukin 1ß (IL-1ß) Standard lyophilized |
| BIO CONJ | Biotin Conjugated Detection Antibody |
| STRP HRP | Streptavidin Horseradish Peroxidase |
| 1X ASY DIL | (1X) Assay Diluent |
| 20X WASH BUF | (20X) Wash Buffer |
| SUB TMB | TMB Substrate |
| SOLN STOP | Stop Solution |
|  | Consult Instructions for Use |
| REF | Catalogue Number |
|  | Expiration Date |
|  | Storage Temperature |

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.