Mouse IL-1β ELISA

REF: KB2063

Ver 5.3

RUO

ELISA for Accurate Quantitation of Mouse IL-1β from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

For Research Purposes Only. 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 KRISHGEN BioSystems is strictly prohibited.

KRISHGEN BioSystems Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400 018.
Tel: (022) 49198700 Email: sales@krishgen.com

Introduction:

Mouse 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:

The Mouse IL-1β ELISA is an enzyme-linked immunosorbent assay for accurate and precise quantitative detection of Mouse IL-1β from samples including serum, plasma, and supernatants from cell cultures. The Mouse IL-1β ELISA is for research use only. Not for diagnostic or therapeutic procedures.

Materials Provided:

1. Microtiter Coated Plate (12 X 8 wells) – 1 no
2. Recombinant Mouse IL-1β Standard – 1 vial
3. Mouse IL-1β Biotin Conjugated Detection Antibody– 1 vial
4. Concentrated Avidin Horseradish Peroxidase – 1 vial
5. Wash Buffer (20X) – 25 ml
6. Assay Diluent (5X) – 10 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
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. Semi-log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer

Storage Information:

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

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.

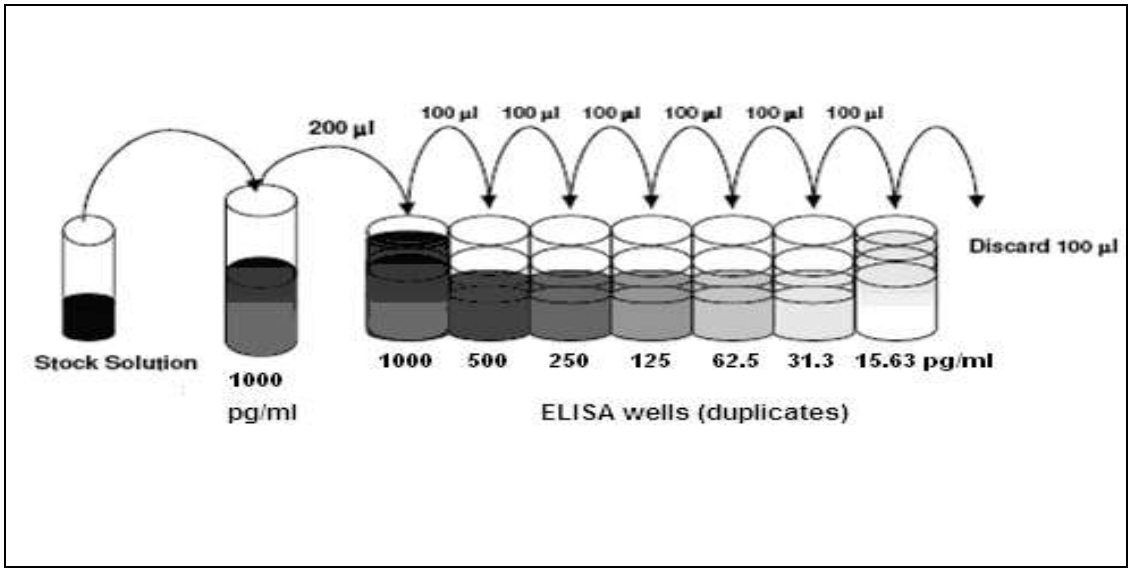
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

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 or triplicate. A standard curve is required for each assay.
2. Add 100µl/well of **Standards** and **Samples** to the plate. Perform two-fold serial dilutions of the 1000pg/ml top standard, either within the plate or in separate tubes. The Mouse IL-1β standard concentrations 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 (0pg/ml). Seal plate and incubate at R.T for 2 hours.



3. 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.

4. Add 100µl of diluted **Detection Antibody** solution to each well, seal plate and incubate at R.T for 1 hour.
5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.
6. Add 100µl/well of **Avidin-HRP** diluted in 1X Assay Diluent. Seal the plate and incubate at R.T for 30 minutes.
7. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.
8. Add 100µl of **TMB Substrate** solution and incubate in the dark at R.T for 15 minutes. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
9. Stop reaction by adding 100µl of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
10. Read absorbance at 450nm within 30 minutes of stopping reaction.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on Semi-log 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.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- **This kit is for in vitro 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 human 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.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2019

THANK YOU FOR USING KRISHGEN PRODUCT!