GENLISA® Mouse Interleukin 1Beta (IL-1BETA / IL1B) ELISA

REF : KB2063

Ver 6.3

RUO

NIBSC Calibrated Assay

*The standards used in this kit are calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK.
1 ng of supplied standard equals 1009 U of 93/668 NIBSC-standard. Please note that the calibration is lot specific.

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 :	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Operating Instructions

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 Krishgen Biosystems Private Limited is strictly prohibited.

REF KB2063 Σ 96 tests

Krishgen Biosystems Private Limited
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: tel +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgen.biz / www.krishgenbio.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.

Long Name: Interleukin 1 beta

Entrez Gene IDs: 3553 (Human); 16176 (Mouse); 24494 (Rat); 397122 (Porcine); 403974 (Canine); 100034237 (Equine); 100135556 (Guinea Pig)

Alternate Names: catabolin; IL1 beta; IL-1 beta; IL-1; IL1B; IL-1b; IL1-BETA; IL-1F2; IL1F2IL-1 beta; interleukin 1, beta; interleukin-1 beta; preinterleukin 1 beta; pro-interleukin-1-beta

Intended Use:

The GENLISA® Mouse Interleukin 1Beta (IL-1BETA / IL1B) ELISA is specifically designed for the accurate quantitation of Mouse IL-1β from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing mouse IL-1β react with already coated affinity purified capture anti-mouse IL-1β antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-mouse IL-1β is added leading to formation of a sandwich complex of solid phase antibody-mouse IL-1β-biotin labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. Streptavidin:HRP conjugate is added which binds to Biotinylated Anti-mouse IL-1β complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3', 5, 5' Tetra Methyl Benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader at 450 nm and it is directly proportional to the concentration of Mouse IL-1β present in the samples.

Materials Provided:

1. Anti-mouse IL-1β Antibody Microtiter Coated Plate (12x8 wells) – 1 no.
2. Recombinant Mouse IL-1β Standard, lyophilized (95 ng/ml) – 2 vials
3. Anti-mouse IL-1β Biotin Conjugated Detection Antibody, lyophilized (15 ug/ml) – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (1X) Assay Diluent – 50 ml
6. (20X) Wash Buffer – 25 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 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
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. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Store recombinant **Standard and Biotin Conjugated Detection at 2-8°C**. After reconstitution aliquot recombinant proteins into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
4. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
5. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

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 Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**: dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.
4. **Standards Preparation:** Reconstitute the lyophilized vial with 40 ul of Assay Diluent (1X) to generate a 95 ng/ml. Add 10.53 ul of reconstituted standard (95 ng/ml) with 989.47 of Assay Diluent (1X) to generate 1000 pg/ml standard. Prepare the **Standards** by serially diluting the standard stock solution as per the below table. Thus the Mouse IL-1β Standards concentration are 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml and 15.6 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
95 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 40 ul Assay Diluent (1X)
1000 pg/ml	Standard No.7	10.53 ul Reconstituted Standard + 989.47 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)
15.6 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	Only Assay Diluent (1X)

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Mouse IL-1β High Dose Hook Effect is due to excess of antibody for very high concentrations of Mouse IL-1β present in the sample.
3. Mouse IL-1β concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Mouse IL-1β.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **100 ul** of **Standards** and **Samples** to each well, Seal plate and incubate for 2 hours at Room Temperature (18-25°C).

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

5. Wash plate 4 times with **Wash Buffer (1X)** as mentioned in step 3.

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

7. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.

8. Add **100 ul** 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.

9. Stop reaction by adding **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

10. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

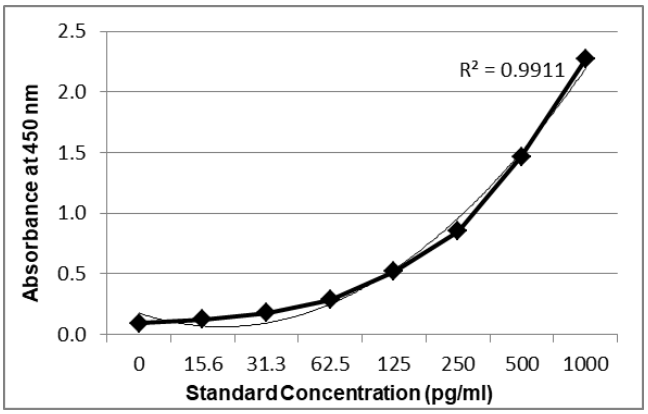
Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight 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.

Typical Data (representative only)

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.093	---	--
15.6	0.125	13.2	84.8
31.3	0.178	29.2	93.2
62.5	0.283	60.5	96.8
125	0.516	132.2	105.8
250	0.851	245.5	98.2
500	1.465	501.0	100.2
1000	2.271	1000.0	100.0

Typical Graph (representative only)



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.

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 IL-1β. The standard used in the kit is calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. 1 ng of supplied standard equals 1009 U of 93/668 NIBSC-standard. Please note that the calibration is lot specific.

Cross Reactivity:
This assay recognizes natural and recombinant human IL-6. The markers listed below were prepared at 50 pg/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant mouse:
IL-1α IL-1ra IL-1 RI/Fc Chimera IL-1 RII/Fc Chimera

Assay Range:

15.6 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 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%

Limitations of Method:

Any diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis. The KB2063 GENLISA® Mouse Interleukin 1Beta (IL-1BETA / IL1B) ELISA is a research use kit only and is not licensed for In-Vitro Diagnostic Use.

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/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- 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.



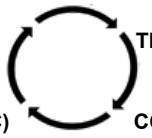
SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) —30 mins / RT Before Use→ 

Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) →  → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

3. Pipette **100 ul Standards** into respective Standard wells.

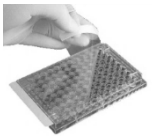
4. Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at room temperature.

6. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

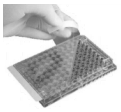
7. Pipette **100 ul** diluted **Biotinylated Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at room temperature.

9. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

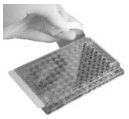
10. Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells.

11. Cover plate  and incubate for 

at room temperature.

12. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

13. Pipette **100 ul TMB Substrate** into each wells.

14. Cover plate  and incubate for 

at room temperature.

15. Pipette **100 ul Stop Solution** into each well.

16. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

LIMITED WARRANTY

Krishgen Biosystems Private Limited 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 Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited 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 Private Limited.

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

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited 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 Private Limited. 2026

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED®, GENLISA®, DHARMAPLEX®, GENBULK®, GENLISA®, KRISHZYME®, KRISHGEN®, KRIBIOLISA®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS PRIVATE LIMITED. ©KRISHGEN BIOSYSTEMS PRIVATE LIMITED. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Anti-mouse IL-1β Antibody Microtiter Coated Plate (12x8 wells)
STD	Recombinant Mouse IL-1β Standard, Lyophilized
BIO CONJ	Anti-mouse IL-1β Biotin Conjugated Detection Antibody, Lyophilized
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