KRISHGEN BioSystems
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GENLISA™ Human High Sensitive Interleukin 1 Beta (IL1B) ELISA

REF: KB1063-HS

Ver 1.0

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 165 U of 86/680 NIBSC-standard. Please note that the calibration is lot specific..

ELISA Set for Accurate Quantitation of Human 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**

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Introduction:

IL-1β is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. IL-1β plays a role in host defense, acute phase reactions, immune response, and hematopoiesis. IL-1β is expressed by T cells, B cells, monocytes, fibroblasts, hepatocytes, endothelial cells, and keratinocytes. Recombinant human IL-1 β is a 20.9 kD protein containing 184 amino acids.

Long Name: Interleukin 1ßeta (IL-1ß)

Entrez Gene IDs: 3553 (Human); 16176 (Mouse); 24494 (Rat); 397122 (Porcine); 281251 (Bovine); 403974 (Canine); 102119749 (Cynomolgus Monkey); 100034237 (Equine); 768274 (Feline); 450200 (Primate); 100008990 (Rabbit)

Alternate Names: Interleukin-1ß, IL-1ß, IL-1F2, Interleukin-1beta, IL-1 beta, IL1b, Interleukin-1 beta, IL-1, IL1-BETA, IL1F2, IL1beta

Intended Use:

Human Interleukin 1 Beta (IL-1B / IL1b) ELISA ELISA is specifically designed for the accurate quantitation of human 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 human IL-1 beta react with already coated affinity purified capture anti- Human IL-1 beta antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IL-1 beta is added leading to formation of a sandwich complex of solid phase antibody-human IL-1 beta-biotin labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. Streptavidin:HRP is added which binds to Biotinylated Anti-human IL-1 beta complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3',5, 5' Tetra Methyl Benzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of Human IL-1 beta present in the samples

Materials Provided:

1. Microtiter Coated Plate (12 x 8 wells) – 1 no
2. Recombinant Human IL-1β Standard lyophilized (1 ug/ml) – 2 vials
3. Human IL-1β Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. (20X) Wash Buffer – 25ml
6. 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. Sterile 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 at 2-8°C**. After 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.

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 temperatures < -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 duplicates. A standard curve is required for each assay.

2. **Standards Preparation:** Reconstitute lyophilized standard vial with 20ul of Distilled water. Let it stand for 15 minutes. Add 5 µl of reconstituted Standard (1 ug/ml) with 495 ul of Assay diluent to generate a 10 ng/ml middle stock solution. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the Standards stock by diluting the middle stock solution as per the below table. Thus, the Human IL-1β standard concentrations are 300 pg/ml, 150 pg/ml, 75 pg/ml, 37.5pg/ml, 18.75pg/ml, 9.38pg/ml and 4.69 pg/ml. Assay Diluent serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
1 ug/ml	Standard, concentrated	Original Standard provided in the Kit + 20ul Distilled water
10 ng/ml	Middle stock	5 ul Original Standard + 495 ul Assay diluent
300 pg/ml	Standard No.7	30 ul Middle stock + 970 ul Assay diluent
150 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay diluent
75 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay diluent
37.5 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay diluent
18.75 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay diluent
9.38 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay diluent
4.69 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay diluent

3. Add 100µl/well of **Standards** and **Samples** to the plate, Seal plate and incubate for 2 hours at 37°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 37°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 37°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 30 minutes at 37°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:

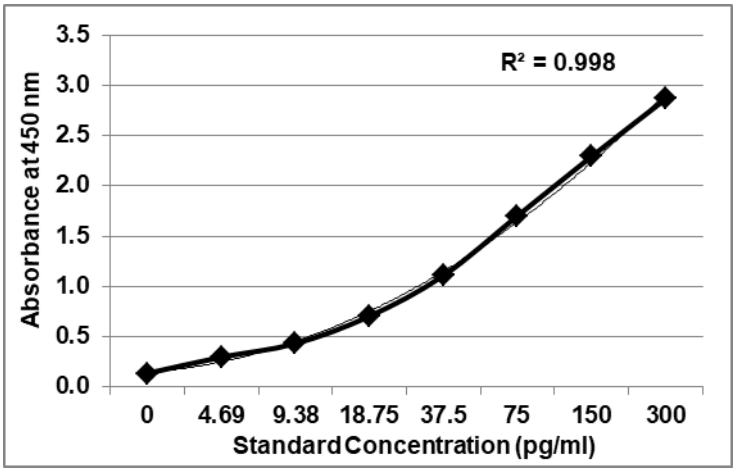
Determine the mean absorbance for each set of duplicates standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight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

Standard Concentration (pg/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.138	--	--
4.69	0.299	4.8	102.0
9.38	0.435	9.1	97.2
18.75	0.706	18.9	100.7
37.5	1.111	37.1	99.0
75	1.704	76.6	102.1
150	2.294	147.1	98.1
300	2.866	302.6	100.9

Typical Graph



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 **3.5 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IL-1β. 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 165 U of 86/680 NIBSC-standard. Please note that the calibration is lot specific.

Cross Reactivity:
This assay recognizes natural and recombinant human IL-1β. 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 human: IL-1α

Recombinant mouse: IL-1α IL-1β

Recombinant rat: IL-1α

Recombinant porcine: IL-1α IL-1β

A sample containing 6250 pg/mL of recombinant rat IL-1β reads as 240 pg/mL (3.8% cross-reactivity). A sample containing 125 pg/mL of recombinant human IL-1β precursor (aa 1-269) reads as 8 pg/mL (6.3% cross-reactivity).

Assay Range:
4.69 pg/ml to 300 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 Human 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 KB1063 GENLISA™ Human Interleukin 1 Beta (IL-1B / 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 at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide 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.

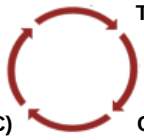
SCHEMATIC ASSAY PROCEDURE

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

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

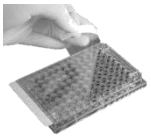
→ Thaw at Room Temperature (18-24ºC) → USE

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

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

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

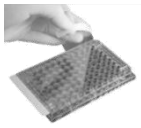
4. Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at 37°C.

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

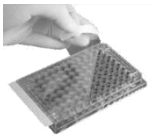
7. Pipette **100 ul** diluted **Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at 37°C.

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

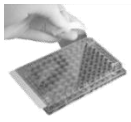
10. Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells

11. Cover plate  and incubate for 

at 37°C.

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 37°C.

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

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

of stopping reaction.

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, 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. 2025

THANK YOU FOR USING KRISHGEN PRODUCT!

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SYMBOLS KEY

MTP	Microtiter Coated Plate (12X8 wells)
STD	Human IL-1β Standard lyophilized
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
ASY DIL	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