Human IL-1α ELISA

REF : KB1062

Ver 4.0

RUO

ELISA Set for Accurate Quantitation of Human IL-1α 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. 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 is strictly prohibited.

KRISHGEN BioSystems| For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: tel +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

Interleukin-1 (IL-1) refers to a group of three polypeptides (interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1ß) and interleukin-1 receptor antagonist (IL-1Ra)), that play a central role in the regulation of immune and inflammatory responses. IL-1α and IL-1β are produced as precursor peptides. Interleukin-1 alpha possesses a wide spectrum of metabolic, physiological, haematopoietic activities, and plays one of the central roles in the regulation of the immune responses. It binds to the interleukin-1 receptor. IL-1α is constitutively produced by epithelial cells.

Intended Use:

Human IL-1α ELISA is specifically designed for the accurate quantitation of human IL-1α 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 no
2. Recombinant Human IL-1α Standard (70 ng/ml) - 2 vial
3. Human IL-1α Biotin Conjugated Detection Antibody - 2 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (20X) Wash Buffer - 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 50ul to 1000ul.
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 recommended temperature.
2. Store recombinant Standard at -20°C. Upon thawing, aliquot recombinant protein and detection antibody 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 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 duplicates. A standard curve is required for each assay.

2. **Standards Preparation:** Upon first use, Thaw and dilute the recombinant protein (70 ng/ml) by adding 3.57µl of standard solution in 496.43µl of Assay Diluent to prepare 0.5ml of the top standard (500pg/ml).Keep the standard for 15 mins with gentle agitation before making further dilutions. Perform serial dilutions by using main stock solution as per the below table. Thus the Human IL-1α Standards concentrations are 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.8 pg/ml and 7.8 pg/ml. Assay Diluent serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
500 pg/ml	Standard No.7	3.57 µl Original Standard + 496.43 µl Assay Diluent
250 pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Assay diluent
125 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Assay diluent
62.5 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Assay diluent
31.25 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Assay diluent
15.8 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Assay diluent
7.8 pg/ml	Standard No.1	250 ul Standard No.2 + 250 ul Assay diluent

3. Add 100 ul of **Standards** and **Samples** to the respective wells.

4. Seal plate and incubate for 2 hours at 37˚C.

5. 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.

6. Add 100 ul of diluted **Detection Antibody** solution to each well, Seal plate and incubate for 2 hours at 37˚C.

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

8. Add 100 ul of diluted **Streptavidin-HRP** solution to each well.

9. Seal plate and incubate for 30 minutes at 37˚C.

10. Wash plate 4 times with **Wash Buffer (1X)** as in step 4. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help to minimize background absorbance.

11. Add 100ul of **TMB Substrate** solution and incubate in the dark for 15-30 minutes at 37°C. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

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

13. 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 be 6.5 pg/ml.

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human IL-1α.

Assay Range:
7.8 pg/ml to 500 pg/ml.

Precision:
Intra-Assay: CV<12%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human IL-1α 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%

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 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 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, 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. 2020

THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Human IL-1α Microtiter Plate (12X8 wells)
STD	Human IL-1α Standard (lyophilized)
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
ASY DIL A	Assay Diluent A
ASY DIL B	Assay Diluent B
AVI HRP DIL	Avidin Horseradish Peroxidase Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instruction For Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature