KRIBIOLISA™ Human IL-6 (Site I / II Binding) ELISA

REF: KBBA60

Ver 1.0

RUO

ELISA for Accurate Quantitation of Human IL-6 (Site I/II) from human serum and plasma

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:

IL-6 is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. IL-6 plays a role in host defense, acute phase reactions, immune response, and hematopoiesis. IL-6 is expressed by T cells, B cells, monocytes, fibroblasts, hepatocytes, endothelial cells, and keratinocytes. Recombinant human IL-6 is a 20.9 kD protein containing 184 amino acids.

The KRIBIOLISA™ Human IL-6 ELISA (Site I/II) is a sandwich ELISA format which uses a cocktail of capture antibodies. These antibodies recognizes and binds interleukin-6 near the binding site I near the N-terminal part of α-helix 1 and the C-terminal part of α-helix 4 of the four-helix bundle, as well as the long loop between α-helices 1 and 2. This binding interface of 68F2 to a major extent overlaps with site I recognized by IL-6R. The antibodies also recognizes and binds to binding site II of human interleukin-6 which interacts with gp130 (IL-6 receptor subunit beta)

Long Name: Interleukin 6

Entrez Gene IDs: 3569 (Human); 16193 (Mouse); 24498 (Rat); 399500 (Porcine); 280826 (Bovine); 403985 (Canine); 102138971 (Cynomolgus Monkey); 100034196 (Equine); 493687 (Feline); 463288 (Primate); 100008733 (Rabbit)

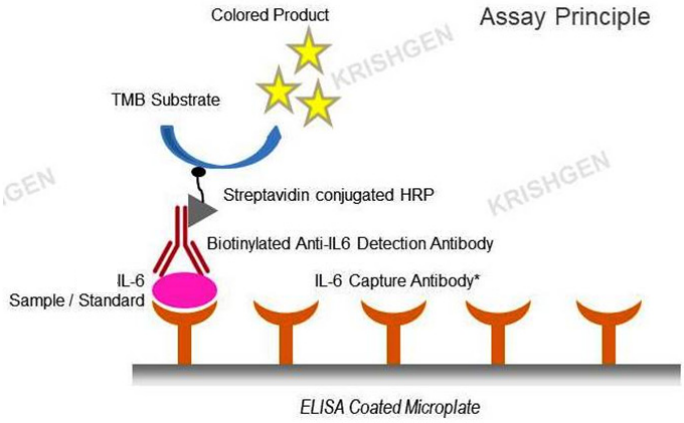
Alternate Names: B-cell differentiation factor; B-cell stimulatory factor 2; BSF2; BSF-2; CDF; CTL differentiation factor ; HSF; hybridoma growth factor; IFNB2; IFN-beta-2; IL6; IL-6; Interferon beta-2; interleukin 6 (interferon, beta 2); interleukin BSF-2; interleukin-6; MGI-2A

Intended Use:

The KRIBIOLISA™ Human IL-6 (Site I/II Binding) ELISA is specifically designed for the accurate quantitation of human IL-6 from human serum and plasma. It is ready-to-use, accurate, and sensitive.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing human IL-6 react with already coated affinity purified capture anti-human IL-6 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IL-6 is added leading to formation of a sandwich complex of solid phase antibody-human IL-6-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-human IL-6 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-6 present in the samples



Materials Provided:

1. Microtiter Coated Plate (12 x 8 wells) - 1 no.
2. Recombinant Human IL-6 Standard lyophilized (1ug/ml) - 2 vials

3. Human IL-6 Biotin Conjugated Detection Antibody - 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (20X) Wash Buffer - 25 ml
6. (1X) Assay Diluent - 50 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 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. Store main kit components at 2-8°C.
2. Store recombinant **Standard at 2-8°C**. Upon reconstituting, aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements. Do not freeze-thaw for more than two times to ensure no loss of activity.
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.

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:** Reconstitute the lyophilized vial with 20 ul of Distilled water to generate a 1 ug/ml. Dilute 5 ul of original Standard (1 ug/ml) with 495 ul of Assay diluent (1X) to generate a 10 ng/ml middle stock solution. Perform serial dilutions by using middle stock solution as per the below table. Thus the Human IL-6

   Standards concentration are 400 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 12.5 pg/ml and 6.25 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
1 ug/ml (Lyophilized)	Standard Main stock	Original Standard. Reconstitute in 20 ul of Distilled water
10 ng/ml	Middle stock	5 ul Original Standard + 495 ul Assay Diluent (1X)
400 pg/ml	Standard No.7	20 ul Middle Stock + 480 ul Assay Diluent (1X)
200 pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Assay Diluent (1X)
100 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Assay Diluent (1X)
50 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Assay Diluent (1X)
25 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Assay Diluent (1X)
12.5 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Assay Diluent (1X)
6.25 pg/ml	Standard No.1	250 ul Standard No.2 + 250 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	500 ul Assay Diluent (1X)

3. Add **100 ul** of prepared **Standards** and **Samples** to the plate.

4. Seal plate and incubate for 2 hours at Room Temperature (18-25°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.

7. Seal plate and incubate for 1 hour at Room Temperature (18-25°C).

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

9. Add **100 ul** of diluted **Streptavidin:HRP** solution to each well, seal plate and incubate for 15 minutes at Room Temperature (18-25°C).

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

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

12. Stop reaction by adding **100 ul** 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:

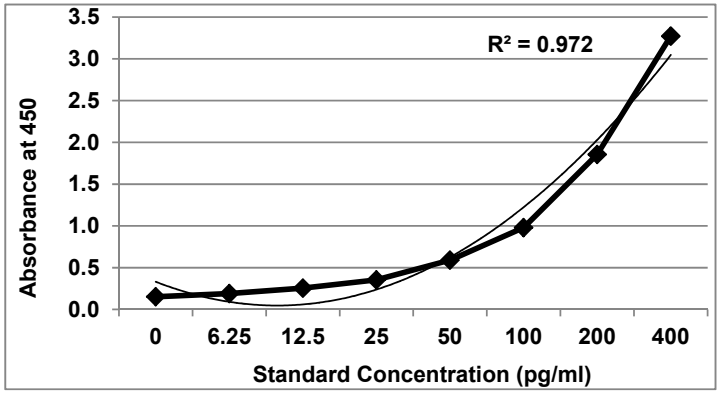
Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight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

Typical Data

Standard Concentration (pg/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.153	--	--
6.25	0.190	5.0	80.6
12.5	0.255	13.3	106.2
25	0.356	25.3	101.4
50	0.590	52.3	104.6
100	0.979	96.7	96.7
200	1.855	201.6	100.8
400	3.273	399.7	99.9

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 **5 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IL-6. These antibodies recognizes and binds interleukin-6 near the binding site I near the N-terminal part of α-helix 1 and the C-terminal part of α-helix 4 of the four-helix bundle, as well as the long loop between α-helices 1 and 2. This binding interface of 68F2 to a major extent overlaps with site I recognized by IL-6R. The antibodies also recognizes and binds to binding site II of human interleukin-6 which interacts with gp130 (IL-6 receptor subunit beta)

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 human:

IL-1alpha	IL-1beta	IL-2	IL-3	IL-4	IL-7	IL-8	IL-10
GM-CSF	IFN alpha	IFN gamma	LIF	MIP-1alpha	MIP-1beta	MCP-1	OSM
RANTES	TGF beta	TNF alpha	TNF beta				

Very low cross reactivity was observed with G-CSF.

Assay Range:
6.25 pg/ml to 400 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-6 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%

Reference Values:
Serum samples from 34 healthy individuals with low CRP levels were evaluated for IL-6 using this ELISA kit. The samples had results in range between 0 to 50 pg/ml. 31 samples obtained value below 17 pg/ml.

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 KB1068 GENLISA Human IL-6 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/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.

Cited References:

Association of elevated IL-6 with poor glycemic control in periodontitis patients
V Kalaivani, YP Kumar, K Rajapandian… - …, 2023 - f1000research.com
… subjected to ELISA for IL-6 using krishgen human IL-6 ELISA kit as per manufacturer's instruction. Descriptive and inferential statistics were used using SPSS software. …

Efficacies of vitamin D and omega-3 polyunsaturated fatty acids on experimental endometriosis
A Akyol, M Şimşek, R İlhan, B Can, M Baspinar… - Taiwanese Journal of …, 2016 - Elsevier
… IL-6, IL-8, VEGF, and TNF-α levels were measured using an enzyme-linked … IL-6 ELISA, Krishgen Biosystems, Mumbai, India; rat IL-8, Eastbiopharm, Hangzhou, China; rat VEGF ELISA …

SCHEMATIC ASSAY PROCEDURE

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

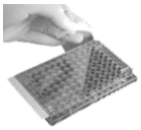
Components (2-8ºC) —30 mins, RT Before Use→  Thaw at Room Temperature (18-24ºC) → USE NOW

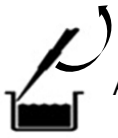
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

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

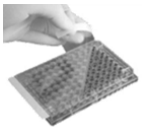
3.  Pipette **100 ul Standards** into respective Standard wells.

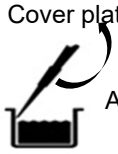
4.  Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for  at room temperature.

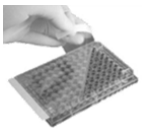
6.  Aspirate and wash wells 5 times with **Wash Buffer (1X).**

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

8. Cover plate  and incubate for  at room temperature.

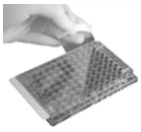
9.  Aspirate and wash wells 5 times with **Wash Buffer (1X).**

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 5 times with **Wash Buffer (1X).**

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

16. Cover plate  and incubate **for**  at room temperature.

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

18. Read absorbance at 450nm with a  microplate reader within  of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Zero Std Zero Std			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Standard No.6 Standard No.6			
1H 2H	Standard No.7 Standard No.7			
3A 4A	Sample			
3B 4B	Sample			
3C 4C	Sample			

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 Products; 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 Products 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 Products 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 Products. 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. 2023

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Human IL-6 Microtiter Plate (12x8 wells)
STD	Human IL-6 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	Catalog Number
	Expiration Date
	Storage Temperature