

# Human IL-6 GENLISA™ ELISA

**REF:** KB1068

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 125 U of 89/548 NIBSC-standard. Please note that the calibration is lot specific.

ELISA for Accurate Quantitation of Human IL-6 from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

|   |                       |   |                                |
|---|-----------------------|---|--------------------------------|
| <b>RUO</b>  | For Research Use Only | <b>REF</b>  | Catalog Number                 |
|  | Store At              | <b>LOT</b>  | Batch Code                     |
|  | Manufactured By       |  | Biological Risk                |
|  | Expiry Date           |  | Consult Operating Instructions |

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**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.

**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)

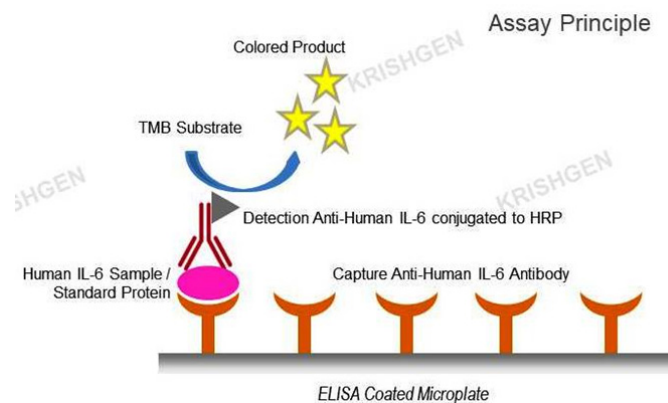
**Alternate Names:** B-cell differentiation factor; B-cell stimulatory factor 2; BSF2; BSF-2; CDF; CTL differentiation factor ; HSF; hybridoma growth factor; IFNB2; IFN-beta-2; IL6; IL-6; Interferon beta-2; interleukin 6 (interferon, beta 2); interleukin BSF-2; interleukin-6; MGI-2A

**Intended Use:**

The GENLISA™ Human IL-6 ELISA is specifically designed for the accurate quantitation of human IL-6 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-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.

*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:** 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)                                   |

- Add **100 ul** of **Standards** and **Samples** to the plate. Seal plate and incubate for 2 hours at Room Temperature (18-25°C).
- Aspirate and wash plate 5 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.
- Add **100 ul** of diluted **Detection Antibody** solution to each well, seal plate and incubate for 1 hour at Room Temperature (18-25°C).
- Wash plate 5 times with **Wash Buffer (1X)** as in step 4.
- Add **100 ul** of diluted **Streptavidin:HRP** solution to each well, seal plate and incubate for 15 minutes at Room Temperature (18-25°C).
- Wash plate 5 times with **Wash Buffer (1X)** as in step 4.
- 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.
- Stop reaction by adding **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
- Read absorbance at 450 nm within 30 minutes of stopping reaction.

#### Calculation of Results:

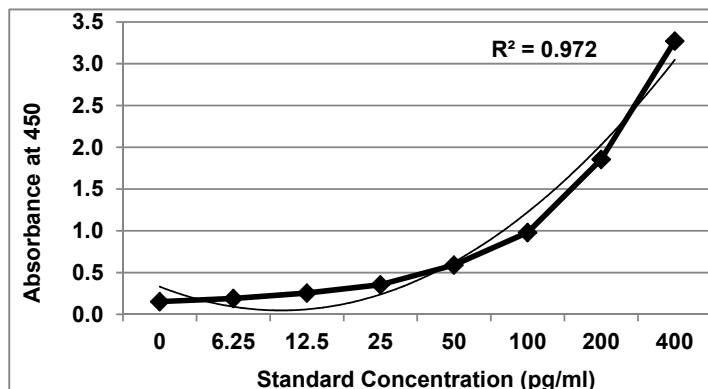
Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight 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. 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 125 U of 89/548 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 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&lt;10%

Inter-Assay: CV&lt;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 Baspınar... - 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 ...

Formulation and evaluation of nano lipid formulation containing CNS acting drug: molecular docking, in-vitro assessment and bioactivity detail in rats

M Alam, AK Najmi, I Ahmad, FJ Ahmad... - Artificial cells ..., 2018 - Taylor & Francis

... BDNF Elisa kit was purchased from Ray Biotech (Norcross GA). TNF- $\alpha$  Elisa kits and IL-6 Elisa kits were purchased from Krishgen BioSystems. All other chemicals and reagents were of ...

International Journal of Medical Stud

BP Panda, SDA Munshi, S Gup - academia.edu

... IL6 activity was tested by using Human IL6 ELISA kit. Analysis of IL-6: Analysis of IL6 by Human IL6 ELISA kit was carried out as per the manufacture instruction (Krishgen Biosystem, ...

Non invasive Assessment of Interleukin 6 and its Clinico-pathologic correlation in patients with Oral Squamous cell carcinoma.

ST Yesupatham, S Arokiyaswamy, AM SM - Medica, 2022 - medicainnovatica.org

... kits by Krishgen BioSystems. The Human interleukin-6 in the micro titer wells interacts with Human interleukin-6 biotin conjugated antibody and the enzyme which was washed using ...

Thymoquinone loaded solid lipid nanoparticles demonstrated antidepressant-like activity in rats via indoleamine 2, 3-dioxygenase pathway

M Alam, S Zameer, AK Najmi, FJ Ahmad... - Drug ..., 2020 - thieme-connect.com

... BDNF Elisa kit was purchased from Ray Biotech (USA). TNF- $\alpha$  Elisa kits and IL-6 Elisa kits were purchased from Krishgen BioSystems. All other chemicals and reagents were of ...

Immunomodulatory effect of rutin, catechin, and hesperidin on macrophage function

A Ganeshpurkar, A Saluja - Indian Journal of Biochemistry and ..., 2020 - op.niscpr.res.in

... ELISA kits for TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were purchased from Krishgen Biosystems, USA. N-... coli) were determined by using Krishgen ELISA based kits as per manufacturer's instructions...

Exploring the possible mechanism involved in the anti-nociceptive effect of  $\beta$ -sitosterol: modulation of oxidative stress, nitric oxide and IL-6

K Kaur, L Singh, A Kaur, R Bhatti - Inflammopharmacology, 2023 - Springer

... Interleukin-6 (IL-6) ELISA kit was purchased from Krishgen Biotech (India). All the other reagents ... Serum IL-6 estimation was done by using commercially available ELISA kits (Krishgen ...

L-theanine, a component of green tea prevents 3-nitropropionic acid (3-NP)-induced striatal toxicity by modulating nitric oxide pathway

S Jamwal, P Kumar - Molecular neurobiology, 2017 - Springer

... IL-6 immunoassay kit (KRISHGEN BioSystem, USA). The quantikine rat TNF-  $\alpha$ , IL-1 $\beta$ , ... IL-6 immunoassay is a 4.5-h solid-phase ELISA designed to measure rat TNF-  $\alpha$ , IL-1 $\beta$ , and IL-6. It ...


Asiatic acid inhibits intracellular Shigella flexneri growth by inducing antimicrobial peptide gene expression

P Maitra, P Basak, K Okamoto, S Miyoshi... - Journal of Applied ..., 2023 - academic.oup.com

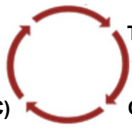
... ELISA was performed to check IL-8, IL-6, and cathelicidin secretion. The antibacterial effect ... ELISA was performed to examine the release of (d) IL-6 and (e) IL-8 in media of control, ...


**SCHEMATIC ASSAY PROCEDURE**


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

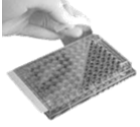

Components (2-8°C)  $\xrightarrow[RT \text{ Before Use}]{30 \text{ mins}}$   Thaw at Room Temperature (18-24°C)  $\longrightarrow$  USE NOW


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


Kit Components  $\xrightarrow[\text{AVOID}]{\text{COOL (2-8°C)}}$    $\xrightarrow[\text{AVOID}]{\text{THAW (22±4°C)}}$  NO CHANGE IN RESULTS

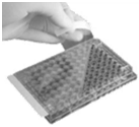

3.  Pipette **100 ul Standards** into respective Standard wells.


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


5.  Cover plate and incubate for  at room temperature.

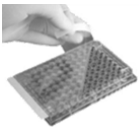

6.  Aspirate and wash wells 5 times with **Wash Buffer (1X)**.


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<br>2A | Zero Std<br>Zero Std           |                     |                 |                            |
| 1B<br>2B | Standard No.1<br>Standard No.1 |                     |                 |                            |
| 1C<br>2C | Standard No.2<br>Standard No.2 |                     |                 |                            |
| 1D<br>2D | Standard No.3<br>Standard No.3 |                     |                 |                            |
| 1E<br>2E | Standard No.4<br>Standard No.4 |                     |                 |                            |
| 1F<br>2F | Standard No.5<br>Standard No.5 |                     |                 |                            |
| 1G<br>2G | Standard No.6<br>Standard No.6 |                     |                 |                            |
| 1H<br>2H | Standard No.7<br>Standard No.7 |                     |                 |                            |
| 3A<br>4A | Sample                         |                     |                 |                            |
| 3B<br>4B | Sample                         |                     |                 |                            |
| 3C<br>4C | Sample                         |                     |                 |                            |

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## SYMBOLS KEY

|   |  |
|---|--|
|    | Human IL-6 Microtiter Plate (12X8 wells) |
|    | Human IL-6 Standard lyophilized          |
|    | Biotin Conjugated Detection Antibody     |
|    | Streptavidin Horseradish Peroxidase      |
|    | (1X) Assay Diluent                       |
|    | (20X) Wash Buffer                        |
|    | TMB Substrate                            |
|   | Stop Solution                            |
|  | Consult Instructions for Use             |
|  | Catalog Number                           |
|  | Expiration Date                          |
|  | Storage Temperature                      |