Rat IL-6 GENLISA™ ELISA

REF KB3068

Ver5.4

RUO

ELISA Set for Accurate Quantitation of Rat IL-6 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:

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and may play a central role in host defense mechanisms. The abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with autoantibody production in patients with cardiac myxoma. Since then, IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases. Measurement of IL-6 levels in serum and other body fluids thus provides more detailed insights into various pathological situations.

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)

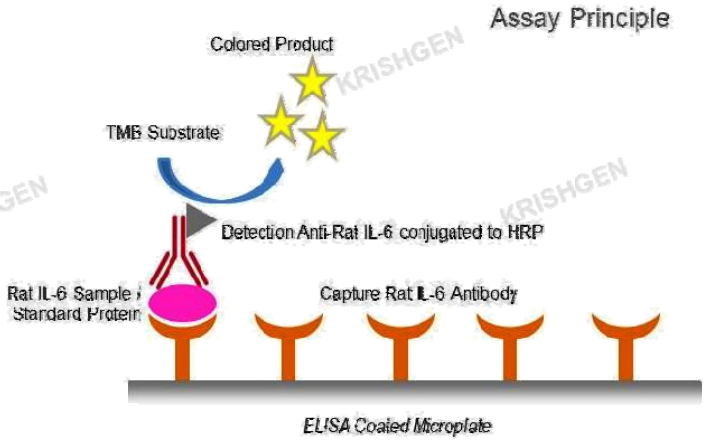
Alternate Names: B-cell differentiation factor; B-cell stimulatory factor 2; BSF2; BSF-2; CDF; CTL differentiation factor ; HSF; hybridoma growth factor; IFNB2; IFN-beta-2; IL6; IL-6; Interferon beta-2; interleukin 6 (interferon, beta 2); interleukin BSF-2; interleukin-6; MGI-2A

Intended Use:

The GENLISA™ Rat IL-6 ELISA is specifically designed for the accurate quantitation of Rat 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 rat IL-6 react with already coated affinity purified capture anti-Rat IL-6 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-Rat IL-6 is added leading to formation of a sandwich complex of solid phase antibody-Rat 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-Rat 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 Rat IL-6 present in the samples



Materials Provided:

1. Microtiter Coated Plate (12 x 8 wells) - 1 no
2. Recombinant Rat IL-6 Standard (230 ng/ml; lyophilized) - 2 vials
3. Rat IL-6 Biotin Conjugated Detection Antibody (lyophilized) - 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (20X) Wash Buffer - 25 ml
6. (1X) Assay Diluent - 50 ml

7. Rat IL-6 Biotin Conjugated Detection Diluent - 10 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. 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. Store main kit components at 2-8°C.
2. Store recombinant Standard and Detection Antibody at 2-8°C. Upon reconstitution, aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements.
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 40 ul of Assay Diluent (1X) to generate a 230 ng/ml. Dilute 20 ul of original Standard (230 ng/ml) with 440 ul of Assay Diluent (1X) to generate a mid-stock of 10 ng/ml. Do further dilutions as per the below table. Thus, the Rat IL-6 standard concentrations are 8000 pg/ml, 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml and 125 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
230 ng/ml	Standard, lyophilized	Lyophilized Standard provided in the Kit + 40ul Assay Diluent (1X)
10 ng/ml	Mid Stock	20ul of Original reconstituted standard + 440ul of Assay Diluent (1X)
8000 pg/ml	Standard No.7	400 ul Mid stock + 100 ul Assay Diluent (1X)
4000 pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Assay Diluent (1X)
2000 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Assay Diluent (1X)
1000 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Assay Diluent (1X)
500 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Assay Diluent (1X)
250 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Assay Diluent (1X)
125 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 **50 ul** of diluted Biotin conjugated **Detection Antibody** to all wells.

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

5. Seal plate and incubate at 37°C for 2 hours.

6. 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.

7. Add **100 ul** 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 6.

9. Add **100 ul** 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 100 ul 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:

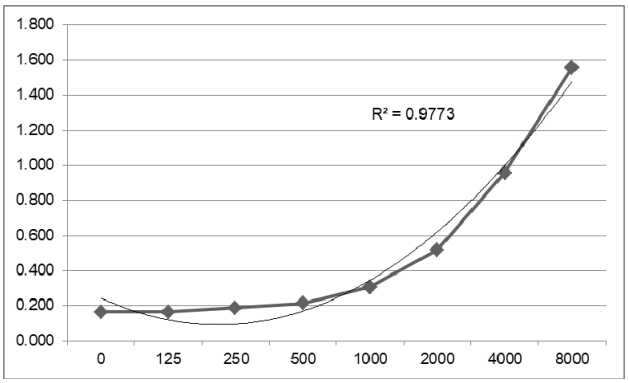
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on semi log graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the 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 like cubic spline or 4PL (2^{nd} order) may be preferred.

Typical Data

Standards (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.164	--	--
125	0.165	123.5	98.8
250	0.190	311.5	124.6
500	0.215	494.7	98.9
1000	0.306	1009.8	101.0
2000	0.518	1987.3	99.4
4000	0.957	4006.9	100.2
8000	1.556	7997.5	100.0

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 **120 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Rat IL-6.

Cross Reactivity:
This assay recognizes natural and recombinant rat IL-6. The factors listed below were prepared at 500 pg/ml in (1X) Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant rat:

CINC-1	GDNF	GM-CSF	IFN-γ	IL-1α	IL-1β	IL-2
IL-4	IL-10	IL-18	β-NGF	PDGF-BB	TNF-α	

Recombinant human:

gp130	IL-6	IL-6 R

Recombinant porcine:
IL-6

Assay Range:
125 pg/ml to 8000 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 Rat 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%

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.

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SYMBOLS KEY

MTP	Rat IL-6 Microtiter Plate (12 x 8 wells)
STD	Rat IL-6 Standard
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
1X ASY DIL	(1X) Assay Diluent
BIO CONJ DIL	Biotin Conjugate 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