

GENLISA™ Human Interleukin 10 (IL-10 / IL10) ELISA

REF : KB1072

Ver 7.5

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 15 U of 93/722 NIBSC-standard. Please note that the calibration is lot specific.

ELISA Set for Accurate Quantitation of Human IL-10 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. 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.

REF KB1072



96 tests



KRISHGEN BioSystems

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Introduction:

Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (**CSIF**), is an anti-inflammatory cytokine. This cytokine is produced primarily by monocytes and to a lesser extent by lymphocytes. This cytokine has pleiotropic effects in immunoregulation and inflammation. It down-regulates the expression of Th1 cytokines, MHC class II antigens, and stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production.

Long Name: Interleukin 10

Entrez Gene IDs: **3569 (Human)**; 3586 (Human); 16153 (Mouse); 25325 (Rat); 397106 (Porcine); 403628 (Canine); 102133450 (Cynomolgus Monkey); 493683 (Feline); 100135553 (Guinea Pig); 2949786 (Viral)

Alternate Names: CSIF, CSIFMGC126450, Cytokine synthesis inhibitory factor, GVHDS, IL-10, IL-10MGC126451, IL10, IL10A, TGIF, interleukin 10, interleukin-10

Intended Use:

GENLISA™ Human Interleukin 10 (IL-10 / IL10) ELISA is specifically designed for the accurate quantitation of human IL-10 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-10 react with already coated affinity purified capture Anti-Human IL-10 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IL-10 is added leading to formation of a sandwich complex of solid phase antibody-human IL-10-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-10 complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3,3',5,5' Tetra Methyl Benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader at 450 nm and it is directly proportional to the concentration of Human IL-10 present in the samples.

Materials Provided:

1. Anti-Human IL-10 Antibody Coated Microtiter Plate (12 x 8 wells) – 1 no.
2. Recombinant Human IL-10 Standard lyophilized (0.25 ug/ml) – 2 vials
3. Anti-Human IL-10 Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. Streptavidin HRP Diluent – 12 ml
6. Assay Diluent – 50 ml
7. (20X) Wash Buffer – 25 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 at 2-8°**. After reconstitution the recombinant protein should be stored 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: ALL STEPS TO BE PERFORMED AT 37°C.

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**
4. **Standards Preparation:** : Reconstitute the lyophilized vial with 20 ul of Distilled water to generate a 0.25 ug/ml. Dilute 10 ul of reconstituted Standard (0.25 ug/ml) with 240 ul of Assay diluent 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-10 standards are 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.6 pg/ml, and 7.8 pg/ml. Assay Diluent serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
0.25 ug/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 20 ul Distilled water
10 ng/ml	Middle stock	10 ul reconstituted Standard + 240 ul Assay Diluent
500 pg/ml	Standard No.7	50 ul Middle stock + 950 ul Assay Diluent
250 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent
125 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent
62.5 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent
31.25 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent
15.6 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent
7.8 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent
0 pg/ml	Standard No.0	Assay diluent Only

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human IL-10. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human IL-10 present in the sample.
3. Human IL-10 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human IL-10.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Add 100 ul of **Standards** and **Samples** to each well, then add 50 ul of diluted **Biotinylated Detection Antibody** to all wells Seal plate and incubate for 2 hours at 37°C.
3. 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.
4. Add 100 ul of diluted **Streptavidin-HRP** solution to each well, seal plate and incubate for 30 minutes at 37°C.
5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.
6. 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.
7. Stop reaction by adding 100 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
8. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

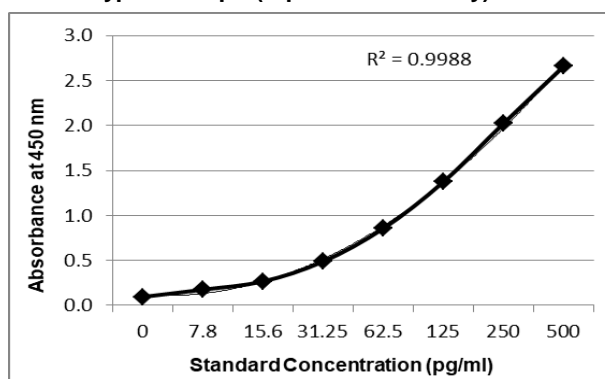
Determine the mean absorbance for each set of duplicates standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight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

Standard Concentration (pg/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.096	1.1	--
7.8	0.176	7.3	93.6
15.6	0.268	14.2	91.1
31.25	0.489	31.4	100.6
62.5	0.856	64.1	102.6
125	1.374	124.7	99.8
250	2.023	247.6	99.0
500	2.656	502.8	100.6

Typical Graph (representative only)



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 **7 pg/ml**.

Specificity:

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IL-10.

Cross Reactivity:

This assay recognizes natural and recombinant human IL-10. 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-10 Rα	IL-10 Rβ/Fc Chimera	IL-22	IL-24		
cotton rat IL-10	feline IL-10	mouse IL-10	porcine IL-10	rat IL-10	viral IL-10

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Calibration:

This Kit has been Calibrated against an International Standard from the National Institute of Biologicals and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. 1 ng of supplied standard equals 15 U of 93/722 NIBSC-standard. Please note that the calibration is lot specific.

Assay Range:

7.8 pg/ml to 500 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-10 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 KB1072 GENLISA™ Human IL-10 ELISA is a research use kit only and is not licensed for In-Vitro Diagnostic Use.

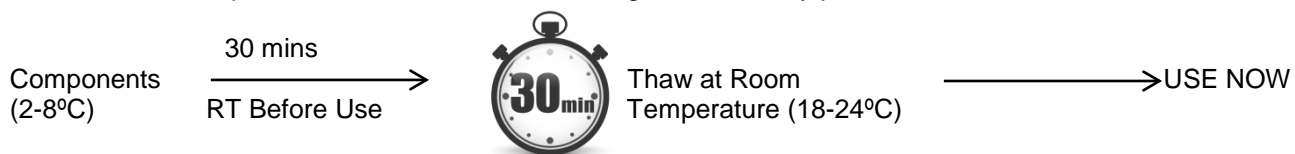
Safety Precautions:

- **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/v) 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.



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



3. Pipette 100 μ l Standards into respective Standard wells.

4. Pipette 100 μ l Samples into the sample wells.

5. Pipette 50 μ l diluted **Biotinylated Detection Antibody** into respective wells.

6. Cover plate and incubate for at 37°C.

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

8. Pipette **100 μ l** of diluted **Streptavidin:HRP** to all wells

9. Cover plate and incubate for at 37°C.

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

11. Pipette **100 μ l TMB Substrate** into each wells

12. Cover plate and incubate for at 37°C.

13. Pipette **100 μ l Stop Solution** into each well.

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

LIMITED WARRANTY

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SYMBOLS KEY

MTP	Anti-Human IL-10 Antibody Coated Microtiter Plate (12 x 8 wells)
STD	Recombinant Human IL-10 Standard, Lyophilized
BIO CONJ	Anti-Human IL-10 Biotin Conjugated Detection Antibody
STRP HRP	Concentrated Streptavidin Horseradish Peroxidase
ASY DIL	Assay Diluent
20X WASH BUF	(20X) Wash Buffer
STRP HRP DIL	Streptavidin:HRP Diluent
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature