






Human TNF- α GENLISA™ ELISA

REF : KB1145

Ver 7.0

RUO

ELISA for Accurate Quantitation of Human TNF- α 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:

Tumor Necrosis Factor- α (TNF- α) is a potent lymphoid factor which exerts cytotoxic effects on a wide range of tumor cells and certain other target cells. Human TNF- α is a 17.4 kD protein containing 157 amino acid residues.

Intended Use:

Human TNF- α ELISA is specifically designed for the accurate quantitation of human TNF- α 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 TNF- α Standard 1ug/ml (lyophilized) – 1 vial
3. Human TNF- α Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (20X) Wash Buffer– 25ml
6. (1X) Assay Diluent – 50ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml
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. Semi-log 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 lyophilized standard at 2-8°C. Upon reconstitution aliquot standards 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 duplicate or triplicate. A standard curve is required for each assay.
2. Standards Preparation: Reconstitute the recombinant protein by adding 20 µl of Distilled water to give a concentration of 1 ug/ml. Keep the standard for 15 mins with gentle agitation before making further dilutions. Add 10 ul of reconstituted standard (1ug/ml) to 990 ul Assay Diluent (1X) to prepare 10ng/ml standard solution. Prepare the Standards stock by diluting the middle stock solution as per the below table. Thus the Human TNF-α Standards concentrations are 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.6pg/ml and 7.8pg/ml, Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
1 ug/ml	Standard, concentrated	Original Standard (lyophilized) + 20ul Distilled water
10 ng/ml	Middle stock	10 ul Original Standard + 990 ul Assay diluent (1X)
500 pg/ml	Standard No.7	50 ul Middle stock + 950 ul Assay diluent (1X)
250 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay diluent (1X)
125 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay diluent (1X)
62.5 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay diluent (1X)
31.25 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay diluent (1X)
15.6 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay diluent (1X)
7.8 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay diluent (1X)

3. Add 100ul/well of **Standards** and **Samples** to the plate, Seal plate and incubate for 2 hours at RT.
4. 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.

5. Add 100µl of diluted **Detection Antibody** solution to each well, Seal plate and incubate for 1 hour at RT.
6. Wash plate 4 times with **Wash Buffer (1X)** as in step 4.
7. Add 100ul of diluted **Streptavidin-HRP** solution to each well, seal plate and incubate for 1 hour at RT.
8. Wash plate 4 times with **Wash Buffer (1X)** as in step 4.
9. Add 100ul of **TMB Substrate** solution and incubate in the dark for 30 minutes at RT. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
10. Stop reaction by adding 100ul 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 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 7 pg/ml.

Specificity:

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human TNF-α.

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 TNF-α 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.




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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Human TNF α Microtiter Plate (12X8 wells)
STD	Human TNF α 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	Catalogue Number
	Expiration Date
	Storage Temperature