

# GENLISA™ Human TNF-Alpha (TNFa / TNA-alpha / TNFA) ELISA

**REF** : KB1145

Ver 7.3

**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 46 U of 12/154 NIBSC-standard. Please note that the calibration is lot specific.

ELISA for Accurate Quantitation of Human TNF- $\alpha$  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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**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: sales1@krishgen.com | <http://www.krishgen.com>

**Introduction:**

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

**Long Name:** Tumor necrosis factor- $\alpha$

**Entrez Gene IDs:** 7124 (Human); 21926 (Mouse); 24835 (Rat); 397086 (Porcine); 280943 (Bovine); 403922 (Canine); 102139631 (Cynomolgus Monkey); 100033834 (Equine); 493755 (Feline); 100009088 (Rabbit)

**Alternate Names:** APC1 protein, Cachectin, Cachetin, DIF, TNF, TNF, monocyte-derived, TNF-A, TNF-alpha, TNF-alpha cachectin, TNFA, TNFATNF, macrophage-derived, TNFG1F, TNFSF1A, TNFSF2, TNFSF2TNF superfamily, member 2, TNFalpha, Tumor necrosis factor ligand superfamily member 2, tumor necrosis factor, tumor necrosis factor (TNF superfamily, member 2), tumor necrosis factor alpha, tumor necrosis factor-alpha.

**Intended Use:**

Human TNF- $\alpha$  GENLISA™ ELISA is specifically designed for the accurate quantitation of human TNF- $\alpha$  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 TNF alpha react with already coated affinity purified capture anti-human TNF alpha antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human TNF alpha is added leading to formation of a sandwich complex of solid phase antibody-human TNF alpha-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 TNF alpha 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 and it is directly proportional to the concentration of Human TNF alpha present in the samples.

**Materials Provided:**

1. Anti-Human TNF alpha Antibody Coated Microtiter Plate (12x8 wells) – 1 no
2. Recombinant Human TNF- $\alpha$  Standard 1ug/ml (lyophilized) – 2 vials
3. Anti-Human TNF- $\alpha$  Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (1X) Assay Diluent – 50 ml
6. (20X) Wash Buffer– 25 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 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. 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 Standard by adding 20 ul of Distilled water to give a concentration of 1 ug/ml. Add 10 ul of reconstituted standard (1 ug/ml) to 990 ul Assay Diluent (1X) to prepare 10 ng/ml Middle stock. Prepare the Standards stock by diluting the middle stock solution as per the below table. Thus the Human TNF-α 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 (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
1 ug/ml	Reconstituted Standard	Lyophilized Standard provided in the kit + 20 ul Distilled water
10 ng/ml	Middle stock	10 ul Reconstituted 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)

**GENLISA™ Human TNF-Alpha (TNFa / TNA-alpha / TNFA)  
ELISA**

Standard Concentration	Standard No	Dilution Particulars
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)
0 pg/ml	Standard No.0	Only Assay diluent (1X)

- Add 100 ul of **Standards** and **Samples** to each well, Seal plate and incubate for 2 hours at RT.
- 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.
- Add 100 ul of diluted **Detection Antibody** solution to each well, Seal plate and incubate for 1 hour at RT.
- Wash plate 4 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 1 hour at RT.
- Wash plate 4 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 RT. 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:**

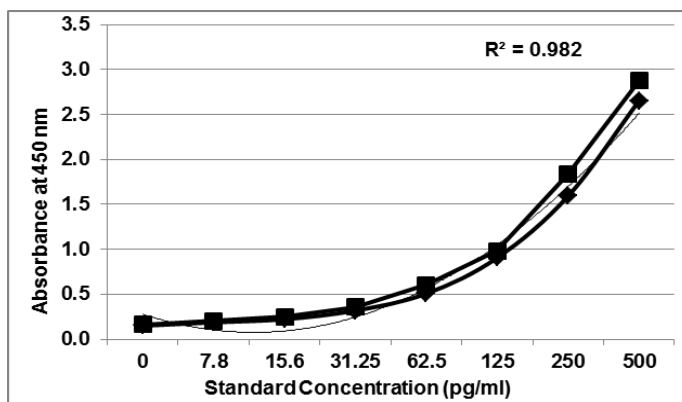
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the 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 2<sup>nd</sup> order is best recommended for automated results.

**Typical Data**

Standard Concentration (pg/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.166	--	--
7.8	0.205	7.5	96.3
15.6	0.247	15.1	97.1
31.25	0.358	32.5	103.8
62.5	0.604	66.9	107.0
125	0.981	118.6	94.9
250	1.842	254.1	101.7
500	2.873	498.5	99.7

**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 be 7 pg/ml.

### Specificity:

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human TNF- $\alpha$ . 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 46 U of 12/154 NIBSC-standard. Please note that the calibration is lot specific.

### Cross-Reactivity:

This assay recognizes natural and recombinant human TNF- $\alpha$ . 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 beta					

### 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- $\alpha$  and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

## GENLISA™ Human TNF-Alpha (TNFa / TNA-alpha / TNFA) ELISA

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.

### 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 KB1145 GENLISA™ Human TNF- $\alpha$  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 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 4 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 4 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 4 times with **Wash Buffer (1X)**.

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

14. Cover plate and incubate for at room temperature.

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

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



## **LIMITED WARRANTY**

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### SYMBOLS KEY

<b>MTP</b>	Anti-Human TNF alpha Antibody Coated Microtiter Plate (12x8 wells)
<b>STD</b>	Recombinant Human TNF-α Standard, Lyophilized
<b>BIO CONJ</b>	Anti-Human TNF-α Biotin Conjugated Detection Antibody
<b>STRP HRP</b>	Concentrated Streptavidin Horseradish Peroxidase
<b>1X ASY DIL</b>	(1X) Assay Diluent
<b>20X WASH BUF</b>	(20X) Wash Buffer
<b>SUB TMB</b>	TMB Substrate
<b>SOLN STOP</b>	Stop Solution
	Consult Instructions for Use
<b>REF</b>	Catalogue Number
	Expiration Date
	Storage Temperature