GENLISA™ Mouse TNF-α ELISA

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Ver 7.4

RUO

ELISA for Accurate Quantitation of Mouse 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 multifunctional cytokine that can exert regulatory and cytotoxic effects on a wide range of normal lymphoid, non-lymphoid, and tumor cells. Mouse TNF-α is a 17.5 KD protein containing 156 amino acid residues.

Long Name: Tumor necrosis factor-α

Alternate Names: TNF, TNF, monocyte-derived, TNF-A, TNF-alpha, TNF-alpha TNFA, TNFATNF, 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:

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

Materials Provided:

1. Anti-Mouse TNF alpha Coated Microtiter Plate (12x8 wells) – 1 no
2. Recombinant Mouse TNF-α Standard, lyophilized (0.5 ug/ml) – 2 vials
3. Anti-Mouse TNF-α Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. (5X) Assay Diluent– 10 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. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store the kit components at 2-8°C.
2. Store recombinant Standard at 2-8°C**.** For long term storage 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. 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 lyophilized Mouse TNF-α standard with 20 ul of distilled water to achieve final concentration 0.5 ug/ml. Dilute 2 ul of Reconstituted Standard (0.5 ug/ml) with 998 ul of Assay diluent (1X) to generate a 1000 pg/ml middle stock solution. Prepare the Standards stock by diluting the middle stock solution as per the below table. Thus the Mouse TNF-α Standards are 450 pg/ml, 225 pg/ml, 112.5 pg/ml, 56.25 pg/ml, 28.13 pg/ml, 14.06 pg/ml and 3.5 pg/ml. “Assay Diluent (1X)” serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
0.5 ug/ml	Reconstituted Standard	Lyophilized Standard provided in the kit + 20 ul Distilled water
1000 pg/ml	Middle stock	2 ul Reconstituted Standard + 998 ul Assay diluent (1X)
450 pg/ml	Standard No.7	450 ul Middle stock + 550 ul Assay diluent (1X)
225 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay diluent (1X)
112.5 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay diluent (1X)
56.25 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay diluent (1X)
28.13 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay diluent (1X)
14.06 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay diluent (1X)
3.5 pg/ml	Standard No.1	250 ul Standard No.2 + 750 ul Assay diluent (1X)
0 pg/ml	Standard No.0	500 ul Assay diluent (1X)

3. Add 100 ul of Standards and Samples to each well, Seal plate and incubate for 2 hours at 37˚C.

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 100 ul of diluted **Detection Antibody** solution to each well, Seal plate and incubate for 1 hour at 37˚C.

6. Wash plate 4 times with **Wash Buffer (1X)** as in step 4.

7. Add 100 ul of diluted **Streptavidin:HRP** solution to each well, seal plate and incubate for 30 min at 37˚C.

8. Wash plate 4 times with **Wash Buffer (1X)** as in step 4. .

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:

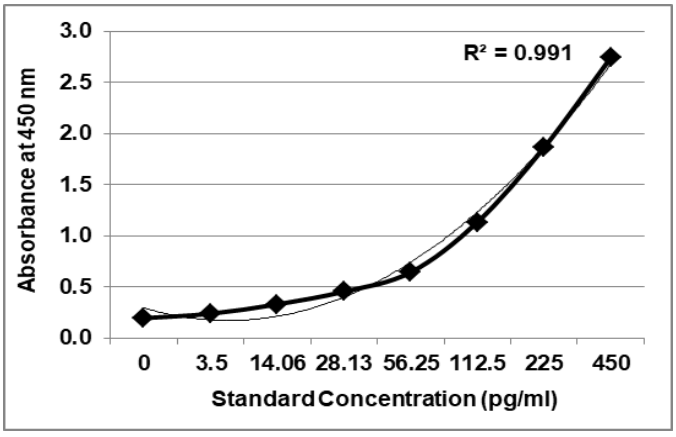
Determine the mean absorbance for each set of duplicates standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight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

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.197	--	--
3.5	0.240	4.1	116.0
14.06	0.332	16.1	114.8
28.13	0.456	30.8	109.6
56.25	0.645	52.7	93.6
112.5	1.135	112.8	100.2
225	1.865	226.3	100.6
450	2.742	449.1	99.8

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 **2.5 pg/ml**.

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

Assay Range:
3.5 pg/ml to 450 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 Mouse 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/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from Mouse 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.

Components (2-8ºC) → 30 mins RT Before Use → 

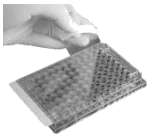
Thaw at Room Temperature (18-24ºC) → USE NOW

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

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

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

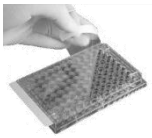
4. Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at 37˚C.

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

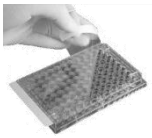
7. Pipette **100 ul** diluted **Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at 37˚C.

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 37˚C.

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 37˚C.

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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THANK YOU FOR USING KRISHGEN PRODUCT!

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SYMBOLS KEY

MTP	Anti-Mouse TNF alpha Coated Microtiter Plate (12x8 wells)
STD	Recombinant Mouse TNF-α Standard, Lyophilized
BIO CONJ	Anti-Mouse TNF-α Biotin Conjugated Detection Antibody
STRP HRP	Concentrated Streptavidin Horseradish Peroxidase
5X ASY DIL	(5X) 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