GENLISA® Mouse Tumor Necrosis Factor Alpha (TNF-A / TNFA) ELISA

REF KB2145

Ver 8.5

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		**Consulting Instructions**

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REF **KB2145** Σ **96 tests**

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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.

Intended Use:

GENLISA® Mouse Tumor Necrosis Factor Alpha (TNF-ALPHA) 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 at 450 nm and it is directly proportional to the concentration of Mouse TNF alpha present in the samples.

Materials Provided:

1. Anti-Mouse TNF-α Coated Microtiter Plate (12x8 wells) – 1 no
2. Recombinant Mouse TNF-α Standard, lyophilized (280 ng/ml) – 2 vials
3. Anti-Mouse TNF-α Biotin Conjugated Detection Antibody (lyophilized, 2.25 ug/ml) – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. Streptavidin:HRP Diluent – 12 ml
6. (1X) Assay Diluent – 25 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. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.

3. Store recombinant **Standard and Detection at 2-8°C**. After reconstitution aliquot recombinant proteins into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
4. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
5. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

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.

1. Bring 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 lyophilized Mouse TNF-α standard with 25 ul of Assay diluent (1X) to achieve final concentration 280 ng/ml. Dilute 7.14 ul of Reconstituted Standard (280 ng/ml) with 992.86 ul of Assay diluent (1X) to generate a 2000 pg/ml Standard Solution. Prepare further Standards by diluting the Standard Solution as per the below table. Thus, the Mouse TNF-α Standards are 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml and 31.25 pg/ml. “Assay Diluent (1X)” serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
280 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the kit + 25 ul Assa
2000 pg/ml	Standard No.7	7.14 ul Reconstituted Standard + 992.86 ul Assay
1000 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay diluen
500 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay diluen
250 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay diluen
125 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay diluen
62.5 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay diluen
31.25 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay diluen

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

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 Mouse TNF-α. High Dose Hook Effect is due to excess of antibody for very high concentrations of Mouse TNF-α present in the sample.
3. Mouse TNF-α 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 Mouse TNF-α.
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, Seal plate and incubate for 2 hours at RT.

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 **Biotinylated Detection Antibody** solution to each well, Seal plate and incubate 2 hours at RT.

5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.

6. Add 100 ul of diluted **Streptavidin:HRP** solution to each well, seal plate and incubate for 30 min at RT.

7. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.

8. 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.

9. Stop reaction by adding 100 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

10. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

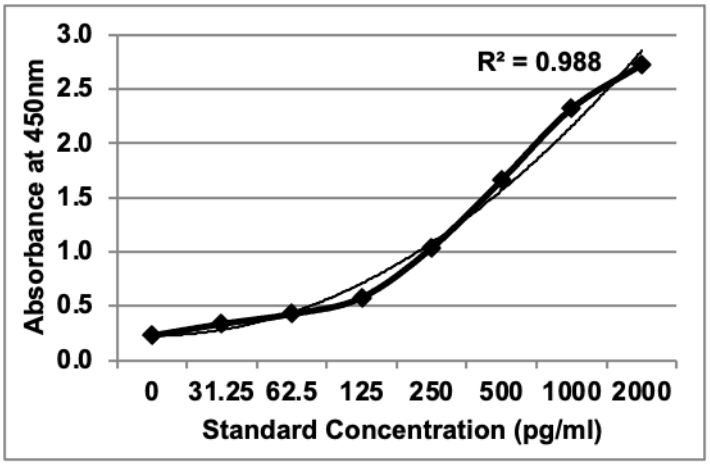
Determine the mean absorbance for each set of duplicates standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight 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 Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.230	--	--
31.25	0.339	30.3	97.0
62.5	0.429	67.7	108.4
125	0.579	117.3	93.9
250	1.035	256.1	102.4
500	1.660	495.6	99.1
1000	2.321	1002.8	100.3
2000	2.725	2000.0	100.0

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 be **31 pg/ml**.

Specificity:

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

Assay Range:

31.25 pg/ml to 2000 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.
- 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

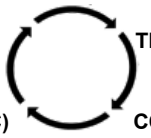
1. Remove all components, 30 minutes before adding into the assay plate.

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) → AVOID → NO CHANGE IN RESULTS



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

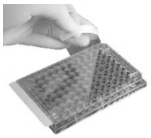
4.  Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at RT.

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

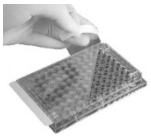
7.  Pipette **100 ul** diluted **Biotinylated Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at RT.

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

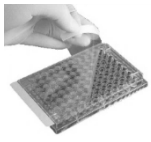
10.  Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells.

11. Cover plate  and incubate for 

at RT.

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 RT.

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

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

of stopping reaction.

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SYMBOLS KEY

	MTP se TNF-α Coated Microtiter Plate (12x8 wells)
	STD hant Mouse TNF-α Standard, Lyophilized
	Anti-Mouse TNF-α Biotin Conjugated Detection Antibody, BIO CONJ
	STRP HRP d Streptavidin Horseradish Peroxidase
	STRP HRP DIL RP Diluent
	1X ASY DIL luent
	20X WASH BUF ffer
	SUB TMB te
	SOLN STOP
	Consult Instructions for Use
	REF Number
	Expiration Date
	Storage Temperature