Mouse Tumor Necrosis Factor a (TNF-a) ELISA

REF :KBH11297

Ver 3.1

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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REF KBH11297 **Σ 96 tests**

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Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

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:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and Standards are pipetted into microwells and Mouse TNF-α present in the sample is bound by the antibodies. After washing Biotin labeled antibody is added and incubated. After washing Streptavidin-HRP is pipetted and incubated. Washing removes any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Mouse TNF-α present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm

Materials Provided:

1. Microtiter Coated Plate (12 X 8 wells) – 1 no
2. Recombinant Mouse TNF-α Standard lyophilized (0.5 ug/ml) – 2 vials
3. Mouse TNF-α Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. (20X) Wash Buffer – 25ml
6. (5X) Assay Diluent– 10ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 1 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator
8. Timer.

Handling/Storage:

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. 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.
4. 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.
2. For Research Use Only.

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 20ul of distilled water to achieve final concentration 0.5 µg/ml. Dilute 2 µl of original 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-alpha Standard concentrations 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 (0pg/ml).

Standard Concentration	Standard No	Dilution Particulars
0.5 ug/ml	Standard (lyophilized)	Original Standard (lyophilized) + 20 ul Distilled water
1000 pg/ml	Middle stock	2 ul Original 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)

3. Add 100ul/well of Standards and Samples to the plate, 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 100ul 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 100ul 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 100ul 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 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:

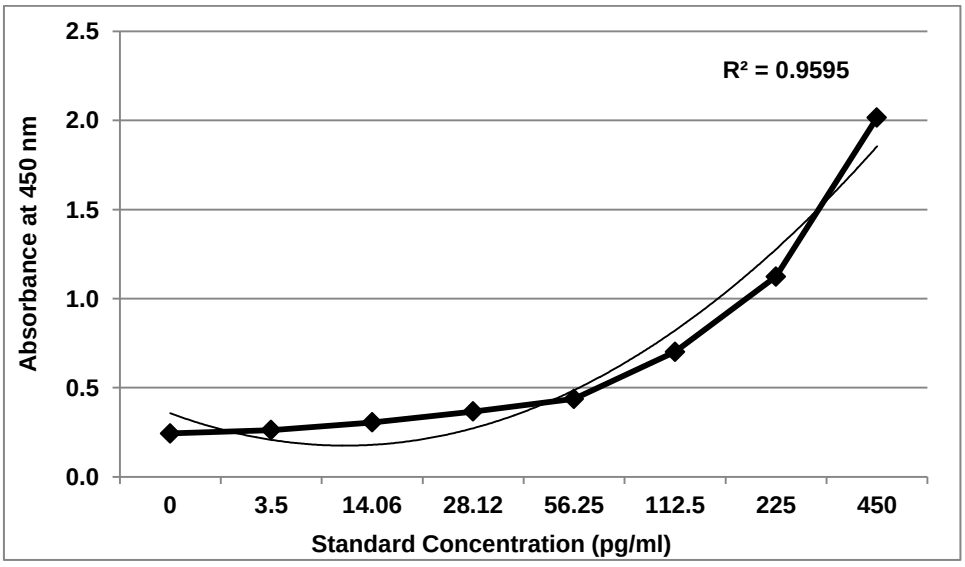
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Mouse TNF-α concentrations, find the unknown's 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 unknown Mouse TNF alpha concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4PL (2nd order) is best recommended for automated results.

Typical Data

Standard Concentration (pg/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.241	0.245	0.243	--	--
3.5	0.269	0.255	0.262	4.0	113.3
14.06	0.324	0.287	0.305	15.3	108.9
28.12	0.368	0.365	0.366	31.2	110.8
56.25	0.430	0.445	0.437	49.4	87.9
112.5	0.700	0.703	0.701	116.7	103.8
225	1.096	1.151	1.124	223.9	99.5
450	1.962	2.071	2.016	450.1	100.0

Typical Graph



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 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.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

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.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
3.5 pg/ml to 450 pg/ml.

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be **2.5 pg/ml.**

Specificity:
This assay has high sensitivity and excellent specificity for detection of TNF-α. No significant cross-reactivity or interference between TNF-α and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of TNF-α and the recovery rates were calculated by comparingthe measured value to the expected amount of TNF-α in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	89-102	97
EDTA Plasma(n=5)	90-99	94
Heparin Plasma(n=5)	90-104	98

Precision:
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of TNF-α and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	87-103%	88-99%	86-98%
EDTA Plasma(n=5)	85-99%	82-100%	83-96%
Heparin Plasma(n=5)	80-98%	82-89%	84-95%

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.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.0 Standard No.0			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Standard No.6 Standard No.6			
1H 2H	Standard No.7 Standard No.7			
3A 4A	Sample			
3B 4B	Sample			

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SYMBOLS KEY

MTP	Mouse TNF-α Microtiter Plate (12X8 wells)
STD	Mouse TNF-α Standard lyophilized
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	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