

EZElisa™ Mouse TNF-Alpha ELISA Kit

Cat #: A-QEK00040

Size: 96wells

Storage: All reagents should be stored as indicated on the component label.

Product information

Introduction

Tumor Necrosis Factor- α (TNF- α) is a potent multifunctional cytokine which can exert regulatory and cytotoxic effects on a wide range of normal lymphoid and non-lymphoid cells and tumor cells. Mouse TNF- α is a 17.5 KD protein containing 156 amino acid residues.

Intended Use

EZElisa™ Mouse TNF-Alpha ELISA Kit 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.

Materials Provided

1. Microtiter Coated Plate (12 X 8 wells) – 1 plate
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. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.
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.

Procedure

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 $\mu\text{g/ml}$. Dilute 2 μl of original Standard (0.5 $\mu\text{g/ml}$) with 998 μl 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 (0 pg/ml).

Standard Concentration	Standard Vial	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 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.

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.

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:



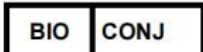
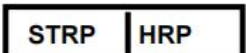
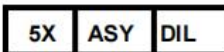
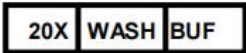

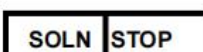




It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

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/w) 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.

SYMBOLS KEY

	Mouse TNF-α Microtiter Plate (12X8 wells)
	Mouse TNF-α Standard lyophilized
	Biotin Conjugated Detection Antibody
	Streptavidin Horseradish Peroxidase
	(5X) Assay Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalogue Number
	Expiration Date
	Storage Temperature

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.