Northwest
Life Science Specialties, LLC

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NWLSS™
Tumor Necrosis Factor Alpha
Murine TNF-α ELISA

Product NWK-TNFAM2
For Research Use Only

ELISA kit for quantification of mouse (murine) Tumor Necrosis Factor Alpha (TNF-α) in biological samples.

Table of Contents

Introduction:

Tumor necrosis factor alpha (TNF-α) is an important cytokine in the innate immune response. It is primarily produced by macrophages but is also produced by other cell types including T-Lymphocytes, Natural Killer (NK), endothelial and neuronal cells. TNF-α is initially produced as a biologically active 26 kDa transmembrane protein, which is subsequently cleaved, principally by TNF-α-converting enzyme (TACE), to release a 17kDa free protein. Free TNF-α is a homotrimer that acts on TNF-α receptors 1 and 2 (TNFR1 and TNFR2) expressed on many different cell types. Both free and transmembrane TNF-α are capable of mediating host responses in acute and chronic inflammatory conditions and have also been shown to be involved in apoptotic cell death, cellular proliferation, differentiation and tumorigenesis. TNF-α has been shown to be a key player in the inflammatory response associated with autoimmune disorders such as rheumatoid arthritis, inflammatory bowel disease (IBD), psoriasis, asthma and others. As such TNF -α blockade has become a common strategy in treating several types of chronic inflammatory disease.

Intended Use:

The NWLSS™ Murine TNF-α ELISA kit is intended to be used for the in vitro quantitative determination of Tumor Necrosis factor Alpha (TNF-α) in murine serum, plasma, cell lysates and cell culture supernatants. The assay will recognize native and recombinant Murine TNF-α.

Test Principle:

The NWLSS™ Murine TNF-α ELISA is a sandwich format Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to murine TNF-α. Samples are pipetted into these wells. Nonbound TNF-α and other components of the sample are removed by washing, after which a biotinylated antibody specific to murine TNF-α is added. In order to quantitatively determine the amount of TNF-α present in the sample, Streptavidin Horseradish Peroxidase (HRP) conjugate is added to each microplate well. After another wash step, TMB-substrate solution is added to each well. Finally, a sulfuric acid stop solution is added and the resulting yellow colored product is measured at 450nm. The amount of TNF-α in the sample can be determined by direct comparison with the standard curve generated in the assay.

Specifications:

Format:	1 X 96 well ELISA presented as 12 X 8 well (6 X 16 well) strips in frame.	
Number of tests:	Triplicate =	24
	Duplicate =	40
Specificity:	Murine (mouse) TNF-α	
Sensitivity:	2.54 pg/mL	
Range:	2.54 pg/mL - 500 pg/mL	

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-Mu TNF-α.	
2 bottles	20X Concentrated Wash Buffer	(25 mL)
1 vial	rMu TNF-α Standard (lyophilized)	(1 Vial)
1 bottle	Standard/Sample Dilution Buffer	(25mL)
1 vial	Secondary Antibody (Lyophilized) (Biotinylated anti-murine TNF-α)	(1 Vial)
1 vial	100X Streptavidin-HRP Conjugate	(150 µL)
1 bottle	Reagent Dilution Buffer	(25mL)
1 bottle	TMB Substrate	(15 mL)
1 bottle	Stop Solution (1 N Sulfuric Acid, H_2SO_4)	(15 mL)
2	Adhesive Plate Covers	(2)

Required Materials Not Provided:

Adjustable micropipettes with disposable tips (5-1000 µL). Multi-channel pipettes are useful and help to reduce intra-sample variability.

Polypropylene tubes.

Serological pipettes.

Deionized water.

Required Instrumentation:
Plate reader with **450 nm** capability (650 nm is required for optional monitoring of color development prior to stopping the reaction).

Warnings, Precautions & Limitations:
Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Substrate solutions must be at room temperature prior to use. Avoid contact of substrate solutions with oxidizing agents and metal.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Storage Instructions:
All kit components of this kit are stable at 2 to 8 ℃. Any unused reconstituted standard should be discarded or frozen at -70 ℃. Standard can be frozen and thawed one time only without loss of immunoreactivity.

Assay Preparation:
1. Determine the number of wells required to assay standards, samples and controls for the appropriate number of replicates. It is recommended that testing be performed in duplicate or triplicate if possible.

2. Create an assay template showing positioning of standards, controls and samples.

3. Bring all samples and reagents to room temperature before use.

4. To avoid condensation, do not open foil pouches containing the microtiter strips until after they have reached room temperature. Next remove the required number of strips and place in the frame supplied.

Return unused wells to the storage bag with desiccant, seal and store at 2-8 ℃.

Reagent Preparation:

Secondary Antibody

1. Reconstitute *Secondary Antibody* by adding 150 µL *Reagent Dilution Buffer* to the vial. Label as 100X Secondary Antibody.

2. Equilibrate *100X Secondary Antibody* to room temperature, mix gently.

3. Mix 20µL of *100X Secondary Antibody* with 1.98 mL *Reagent Dilution Buffer* for each 16 well strip to be assayed. Label as **"Working Secondary Antibody ".**

4. Return the unused reconstituted *100X Secondary Antibody* to the refrigerator.

100X Streptavidin-HRP Conjugate

1. Equilibrate to room temperature, mix gently.

2. Mix 20µL of *100X Streptavidin-HRP Conjugate* with 1.98 mL *Reagent Dilution Buffer* for each16-well strip to be assayed. Label as **"Working Streptavidin-HRP Conjugate**

3. Return the unused *100X Conjugate* to the refrigerator.

Wash Buffer

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.

2. Mix 0.5 volume *20X Wash Buffer* with 9.5 volumes of *deionized water.* Label as **"Working Wash Solution".**

3. Store both the remaining concentrated Wash Buffer and the Working Wash Solution at 4°C in the refrigerator.

TMB Substrate

The TMB Substrate is provided ready to use.

Stop Solution

The Stop Solution is provided ready to use

Sample Handling/Preparation

The rate of degradation of murine TNF-α in various matrices has not been fully investigated. It is beyond the scope of this publication to comment on specific sample processing protocols except to state that sodium citrate, heparin or EDTA are all acceptable forms of anticoagulant for use in harvesting plasma for this assay. It is also recommended that serum or plasma samples be centrifuged and separated from coagulated or packed cells as soon as possible after harvest. Serum and plasma samples should be diluted 4X prior to assay.

Cell lysates can be made by isolating cell samples followed by mechanical homogenization or sonication. Homogenates should be centrifuged and the assay performed on the clarified supernatant. Since the concentration of TNFα in a cell or tissue homogenate will be highly dependent on type of cell or tissue it up to the end-user to optimize the dilutional scheme for their specific sample type.

Standard Curve Preparation:

Reconstitute the murine TNF-α standard to 10 ng/mL by adding 1mL of *Standard/Sample Dilution Buffer* into the standard protein glass vial containing lyophilized murine TNF-α. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution. Use or freeze within 1 hour of reconstitution.

1. Label tubes 1-8 tubes as:
500, 250, 125, 62.5, 31.25, 15.63, 7.81 and zero (0) pg/mL.

2. Add 950 µL ***Standard/Sample Dilution Buffer*** to tube 1 and 500 µL **Standard/Sample Dilution Buffer** to each of tubes 2-8.

3. Add 50 µL ***Reconstituted 10 ng/mL Standard*** to tube 1 and mix well.
Note: Unused reconstituted standard can be frozen at -70 °C and thawed one time only without significant loss of immunoreactivity.

4. Make a serial dilution by transferring 500 µL of 500 pg/mL Standard (tube 1) into tube 2 mixing thoroughly then 500 µL of resulting 250 pg/mL to tubes 3 and so on to create all Standards down to 7.81 pg/mL.

Assay Protocol:

1. Add 100 µL of ***Diluted Standards*** to the appropriate microtiter wells and 100 µL of ***Standard/Sample Dilution Buffer*** to zero wells.

2. Add 100 µLof ***Diluted*** (if necessary) ***Sample*** to each well according to plan.

3. Cover the plate with the plate cover and incubate for 2 hours at 37 °C.

4. Thoroughly aspirate or decant the solution from the wells.

5. Wash wells 3 times as follows: Dispense 300 µL ***Working Wash Solution*** to each well and allow to soak for 1-3 minutes before decanting or aspirating the remaining solution from the wells.

6. Add 100 µL of ***Working Secondary Antibody*** to each well.

7. Cover the plate with the plate cover and incubate for 1 hour at 37 °C.

8. Aspirate or decant the solution from the wells then wash the wells 3 times as previously described in step 5.

9. Add 100 µL ***Working Streptavidin-HRP Conjugate*** to each well.

10. Cover the plate with the plate cover and incubate for 30 minutes at room temperature 37 °C.

11. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times previously described in step 5.

12. Add 100 µL of ***TMB Substrate*** to each well. The highest standard wells and sample wells with high levels of TNF-α should begin to turn blue.

13. Incubate the plate at room temperature for approximately 5-10 minutes. In some cases longer incubation may be necessary.

Note: The incubation time for the TMB substrate is dependent on ambient conditions. The user can adjust this time as necessary by monitoring the development of blue color at 650 nm and applying stop solution when the high standard has reached near maximal absorbance level.

14. After appropriate incubation time, add 100 µL of ***Stop Solution*** to each well. The solution in the wells should change from blue to yellow.

15. Read and record the absorbance of each well at 450 nm within 20 minutes of adding the Stop Solution.

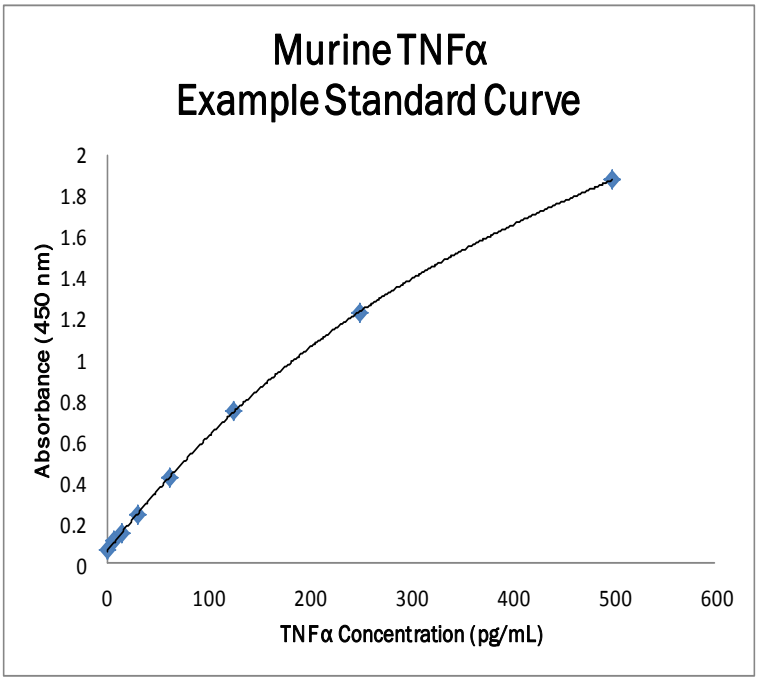
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the TNF-α concentration. Select the best possible fit for the curve obtained. This can typically be done using the software provided with most plate readers. An example curve is shown below.

2. Sample TNF-α is determined by comparing their absorbance at 450 with those of the standard curve.

3. Sample data as read from the standard curve must be multiplied by the dilution factor used.

Note: Samples with an ABS_{450} exceeding that of the highest standard should be additionally diluted with Sample Dilution Buffer and re-assayed in order to avoid erroneous results.



Performance Details:

Crossreactivity:
The following substances were tested and found to have no cross-reactivity:

Human TNF-α
Rat TNF-α

Sensitivity
The lower limit of detection for Murine TNF-α in this assay was calculated by adding three standard deviations to the mean of 12 zero standard replicates and intersecting this value with the standard curve obtained in the same calculation. Sensitivity was calculated to be 2.54 pg/mL.

Precision
Intra-assay CV = 4.43 %
Inter-assay CV = 4.26 %

Accuracy:
Recovery on addition is 94~101% (Average 97.8%)

References:

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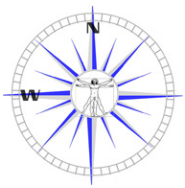
Statement of Limited Warranty:
Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

Notes:



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