

Human TNF- α ELISA Kit

Catalog# UZ-ES00006

Product information

Catalog#	UZ-ES00006
Product Name	Human TNF- α ELISA Kit
Species	Human
Method	Sandwich ELISA
Detection Range	5 - 80 pg/ml
Sensitivity	< 1.0 ng/mL
Sample Type	Serum, plasma, cell culture supernatant, tissue homogenate
Storage	2-8°C, protected from light and moisture
Validity	6 months
Size	96 wells / 48 wells

1. Intended Use

This TNF- α ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of TNF- α in the sample, this TNF- α ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus TNF- α concentration. The concentration of TNF- α in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2. Sample Collection and Storage

1. Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Note: The samples should be centrifuged adequately and no hemolysis or granule was allowed.

3. Materials Required but Not Supplied

1. Standard microplate reader(450nm)
2. Precision pipettes and Disposable pipette tips.
3. 37 °C incubator

4. Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
3. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C)

5. Materials Supplied

Human TNF- α ELISA Kit

Catalog# UZ-ES00006 (cont.)

Product information

Name	96 determinations	48 determinations
Microelisa stripplate	12*8strips	12*4strips
Standard	0.3ml*6tubes	0.3ml*6tubes
Sample Diluent	6.0ml	3.0ml
HRP-Conjugate reagent	10.0ml	5.0ml
20X Wash solution	25ml	15ml
Chromogen Solution A	6.0ml	3.0ml
Chromogen Solution B	6.0ml	3.0ml
Stop Solution	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Note: Standard (S0 $\rightarrow$ S5) concentration: 0,5,10,20,40,80 pg/ml

6. Reagent Preparation

20 $\times$ wash solution: Dilute with Distilled or deionized water 1:20.

7. Assay Procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Add standard: Set Standard wells, testing sample wells. Add standard 50 μ l to standard well.
3. Add Sample: Add testing sample 10 μ l then add 40 μ l of Sample Diluent to testing sample well; Blank well doesn't add anything.
4. Add 100 μ l of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400 μ l) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add chromogen solution A 50 μ l and chromogen solution B 50 μ l to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
7. Add 50 μ l Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
- 8 Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

8. Calculation of Results

Human TNF- α ELISA Kit

Catalog# UZ-ES00006 (cont.)

Product information

This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve. The sensitivity by this assay is 1.0 pg/ml.

9. Storage and Validity

Storage 2-8°C.

Validity six months.

10. Disclaimer

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES. The kit is intended for research use only; any consequences arising from clinical or human-use applications shall be borne solely by the experimenter. Strictly follow the instructions; any deviation is the responsibility of the operator.