Human TNF- ELISA kit

Cat. No.: DEIA4323
Pkg. Size: 96T

Intended use

For quantitative detection of human TNF-α in cell culture supernatants, human plasma (EDTA, heparin and citrate), serum, cerebrospinal fluid, urine, synovial fluid or other body fluids.

General Description

Tumor Necrosis Factor-α (TNF-α) is a non-glycosylated 17.5 kDa, 157 amino acid protein. TNF-α is a potent lymphoid factor and exerts cytotoxic effects on a wide range of tumor cells and other target cells. It is secreted by macrophages, monocytes, neutrophils, T-cells, and NK-cells following their stimulation by bacterial lipopolysaccharides. TNF-α has been suggested to play a pro-inflammatory role and has been detected in synovial fluid of patients with rheumatoid arthritis. Various pathological conditions are associated with the production of high levels of TNF-α. These include septic shock, cachexia (e.g. HIV, tuberculosis, cancer), autoimmune diseases, hepatitis, leukemia, myocardialischaemia, organ transplantation rejection, multiple sclerosis, rheumatoid arthritis, and meningococcal septicemia. Annually, many people die from septic shock syndrome, triggered by TNF-α following complications from an infectious disease. In many cases elevated TNF-α serum levels predict a higher mortality.

Principle Of The Test

This assay employs an antibody specific for human TNF-α coated onto a 96-well plate. Standards, samples and biotinylated anti-human TNF-α are pipetted into the wells. TNF-α present in a sample is captured by the antibody immobilized to the wells and by the biotinylated TNF-α specific detection antibody. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed. Following the second wash step, TMB substrate solution is added to the wells, resulting in color development proportional to the amount of TNF-α bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. 96 Well Plate with 12 Strips. Break apart microtiter test strips each with TNF-α antibody coated single wells Ready for use. 1 frame
2. TNF-α Standard Lyophilized & Stabilized Human TNF-Alpha, Recombinant with the sample diluent volume shown on the label. 4 vials
3. Biotinylated TNF-α antibody Ready for use. 10 mL
4. HRP-Conjugated Avidin Ready for use. 12 mL
5. 20x Washing solution concentrate (sufficient for 1000 mL) Dilute 1:20. 50 mL
6. Dilution buffer Ready for use. 100 mL
7. Stop solution 0,9 N H2SO4 Ready for use. 8 mL
8. TMB-Substrate Ready for use. 8 mL
9. Quality control certificate. 1

Materials Required But Not Supplied
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Multi-channel pipet (25 µL to 350 µL).
4. Adjustable 1-25 mL pipettes for reagent preparation.
5. 100 mL and 1 liter graduated cylinders.
6. Absorbent paper.
7. Distilled or de-ionized water.
8. Log-log graph paper or computer and software for ELISA data analysis.
9. Tubes to prepare standard or sample dilutions.
10. Timer

Storage

Store the complete kit at 2-8°C.

Reagent Preparation

1. Antibody coated plate: Before opening the foil pouch, determine the number of strips required to test the desired number of samples, plus 16 wells needed for running standards and blanks in duplicate. Remove non-used strips from the plate-frame and return them to the foil pouch containing the desiccant for up to 3 months at 2-8°C.
2. Dilution of test standard: Dissolve the lyophilised TNF- standard with Sample Diluent volume shown on the label. TNF-standard is unstable after dissolving. Use immediately or keep on ice if not used within 1 hr after dissolving.
To obtain a standard curve dilute it as follows:
1). Add 300 µL of TNF- standard from kit standard tube containing 1000 pg/mL of TNF-α (Standard tube 1.)
2). Add 150 µL of Sample Diluent to all other 6 dilution tubes. Take 150 µL from the first tube (1000 pg/mL) and start 2-fold serial dilutions in dilution tubes as described in the figure by mixing several times with the pipet in each tube (Total of 7 dilution tubes).
3). 150 µL of sample Diluent in tube 8 serves as zero standard (0 pg/mL).
3. Sample preparation and dilution: Dilution of samples is not required for initial screening. Samples that exceed the measuring range should be diluted in sample diluent serially 1:2, 1:4, or further if necessary, and measured again. The dilution factor must be taken in account when calculating the results.
Dilute and store all samples in tubes or plates made of material with low binding surface, such as polypropylene.
4. Sample collection and storage: Serum, EDTA, heparin or citrate anti-coagulated plasmas, cerebrospinal fluid, urine, synovial fluid, other body fluids and cell culture supernatants are suitable for use in the assay (caution: separate plasma/serum and blood cells within 4 hours after collection, non-separated samples must be kept at 2-8°C). Do not use grossly haemolysed or lipemic specimens. If samples are to be run within 24 hours, they may be stored at 2-8°C; otherwise samples should be stored frozen (at least between -18 to -32°C, but preferably < -70°C). Up to 3 freeze-thaw cycles have no effect on the TNF-α levels of samples. Nonetheless, excessive freeze-thaw cycles should be avoided. Prior to the assay, frozen samples should be thawed as quickly as possible in tap water (18-25°C), do not use 37°C or 56°C water bath for this purpose.
5. Preparation of reagents:
1). Wash Buffer: If the 20x concentrated Wash Buffer contains visible crystals, warm it at 37°C and mix gently until dissolved.
Dilute 1:20 with de-ionized or distilled water (e.g. 25 mL of Wash Buffer Concentrate and 475 mL distilled water to yield 500 mL of 1x Wash Buffer). Check the pH of the diluted wash buffer and adjust to 7.4 if necessary.
2). Vortex mix Biotinylated antibody solution gently before use.
3). Vortex mix peroxidase (HRP) labeled avidin gently before use.

Assay Steps
1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Leave some wells as a reagent blank (2 to 4 wells).

2. Pipette 50 µL of sample and 50 µL of each diluted standard starting from 1000 pg/mL into appropriate wells. Pipette 50 µL of sample diluent to the wells which will be used as a blank. Incubate 1 hr at room temperature without shaking.

3. Wash 5x with 1x Wash Solution (300 µL each)
   To wash manually: Empty plate contents. Use a multi-channel pipette to fill each well with 300 µL of diluted wash buffer, then empty plate contents again. Repeat procedure 4 additional times for a total of FIVE washes. Gently blot plate onto paper towels or other absorbent material. Never let reaction wells dry. Continue to the next step without delay or interruption.
   For automated washing: Aspirate all wells and wash 5 times with 300 µL diluted wash buffer. Blot plate onto paper towels or other absorbent material. Never let reaction wells dry. Continue to the next step without delay or interruption.

4. Promptly add 50 µL of green colored Biotinylated TNF-alpha detection antibody to all wells. Tap the plate gently by hand to homogenize your mixture. Avoid touching to the reaction wells with the pipette tip. Incubate at room temperature for 30 minutes without shaking.

5. Wash 5 times 5x as described in Step 3. Add 50 µL of prepared HRP-conjugated avidin solution (ready to use) to each well. Incubate for 30 minutes at room temperature.

6. Wash 5 times as described in Step 3.

7. Using a multichannel pipette, promptly add 50 µL of TMB ready to use substrate reagent to each well. Incubate for 20 minutes at room temperature in the dark.

8. Add 25 µL of Stop Solution to each well. Read at 450 nm within 15 minutes. Correcting for optical imperfections in the microplates by subtracting A630 nm is recommended, but not an essential procedure.

9. Calculate the mean of reagent blank absorbance values and subtract it from all test well values (standard and test samples). Mean reagent blank absorbance value at 450 nm should be less than 0.200.

10. Calculate your results against standard curve, as outlined below.

**Calculation**

The standard curve must be determined individually for each experiment. Correct the absorbance values of all standards by subtracting from them the mean O.D. value of the reagent blank (B1 = only sample diluent). Calculate the mean absorbance value for each standard from the duplicates.

The standard curve is used to determine the amount of TNF alpha in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding TNF alpha concentration (pg/mL) on the horizontal (X) axis.

Construct the standard curve using graph paper or statistical software.

If samples generate values higher than the highest standard, dilute the samples with sample diluent and repeat the assay. Note that the concentration read from the standard curve must be multiplied by the dilution factor.

**Typical Standard Curve**

The following standard curve is obtained for various concentrations of TNF-α standard over the range of 0 to 1000 pg/mL.

Please note: The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
**Detection Range**

0-1000 pg/mL

**Sensitivity**

<15 pg/mL

**Specificity**

No cross reactivity was observed with the following recombinant human proteins: IL-1β, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, TARC

**Reproducibility**

intra-assay: 6%.

Inter-assay: 4%.

**Precautions**

1. When not in use, kit components should be kept refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags.
3. Once the desired number of strips has been removed, immediately return unused strips to the bag, reseal the bag and store at 2-8°C to maintain plate integrity. Protect from humidity.
4. Samples should be collected in pyrogen/endotoxin-free tubes.
5. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
6. When possible, avoid the use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
7. It is recommended that all standards, controls and samples are run in duplicate. Samples that are >700pg/mL should be diluted with Sample Diluent.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. Do not use reagents past their expiration date.
11. Read absorbencies within 20 minutes of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash buffer must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
14. Because TMB substrate solution is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB substrate solution and metal, or color may develop.

### Analyte Gene Information

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<tr>
<th>Gene Name</th>
<th>TNF tumor necrosis factor [ Homo sapiens ]</th>
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<td>Official Symbol</td>
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<td>Synonyms</td>
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<td>Function</td>
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### REFERENCES