

# Human Tumor Necrosis Factor Alpha ELISA

Cat. No. 770221

IVD

The **DIAMED EUROGEN TNF- $\alpha$  ELISA** is an enzyme immunoassay for the quantitative determination of Human Tumor Necrosis Factor Alpha (TNF- $\alpha$ ) in serum, other biological fluids or culture supernatant.

## PRINCIPLE

Microtiterstrips coated with anti-TNF- $\alpha$  monoclonal antibodies are incubated with standards and test samples. During this step, TNF- $\alpha$  present in the standards and samples will bind with the immobilized antibodies.

After removal of unbound TNF- $\alpha$  and other proteins by a washing procedure, biotinylated anti-TNF- $\alpha$  monoclonal antibodies are incubated in the strips.

These labeled antibodies bind with the TNF- $\alpha$  molecules captured in the previous step.

Again, a washing procedure is performed to remove excess of biotinylated antibodies.

In a third step, streptavidin conjugated Horse Radish Peroxidase (HRP) is added to the wells. Streptavidin binds biotin of the biotinylated antibodies with a high affinity.

After a third washing step the microtiterstrips are incubated with a substrate solution containing hydrogen peroxide and a tetramethylbenzidine buffer solution. A blue colour develops in proportion to the amount of TNF- $\alpha$  present in the standards and/or unknown samples. This enzymatic reaction is ended by the addition of 2 N H<sub>2</sub>SO<sub>4</sub> and the optical density (absorbance) of the solution in each well is determined at 450 nm.

A standard curve is obtained by plotting each absorbance value versus the corresponding standard value (pg/ml). The concentration of TNF- $\alpha$  in the unknown samples is determined by interpolation from this standard curve.

## REAGENTS

- Standards.** 3 vials containing +/- 10 ng (the exact concentration is indicated on the vials) lyophilized human TNF- $\alpha$ . The content of these vials has to be reconstituted with Zero Standard/Sample Diluent or alternative matrices for the determination of TNF- $\alpha$  in cell culture supernatans or non-serum samples (e.g. spinal fluid, urine, synovial fluid ...). The standard is calibrated against NIBSC International Standard for TNF- $\alpha$  (natural human type) 88/786.
- Zero standard/Sample diluent 1 (for determination of TNF $\alpha$  in serum samples).** 1 vial containing 10 ml of horse serum, to be used as zero standard and for dilution of human serum samples. Contains antimicrobial agents.
- Zero standard/Sample diluent 2 (for determination of TNF $\alpha$  in culture supernatant or other biological fluids).** 1 vial containing 25 ml of a buffer solution, to be used as zero standard and for dilution of samples. Contains antimicrobial agents and an inert green dye.
- Coated Microtiterstrips.** 1 microtiterplate (12 x 8-well strips) coated with anti-TNF- $\alpha$  monoclonal antibodies.
- Washing buffer.** 1 vial containing 100 ml 20 x concentrated washing solution. Contains antimicrobial agents.
- Biotin-anti-TNF- $\alpha$  Conjugate.** 1 vial containing 15 ml of biotinylated anti-TNF- $\alpha$  monoclonal antibodies. Contains antimicrobial agents and an inert red dye.
- Enzyme conjugate (Streptavidin-HRP).** 1 vial containing 15 ml of streptavidin conjugated Horse Radish Peroxidase. Contains antimicrobial agents and an inert red dye.
- Chromogen Solution** , 1 vial, containing 25 ml buffer with peroxide and tetramethylbenzidine.
- Stopping Solution.** 1 vial containing 12 ml 2 N H<sub>2</sub>SO<sub>4</sub>.

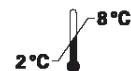
## MATERIALS REQUIRED BUT NOT SUPPLIED.

- Precision micropipettes, including tips of 50 and 125  $\mu$ l capacity.
- Clean standard laboratory volumetric glassware.
- Microtiterplate reader capable of measuring absorbances at 450 nm.

## WARNINGS AND PRECAUTIONS FOR USERS.

- This ELISA-kit is intended for in vitro diagnostic use only.
- Human blood components used in the preparation of the standard sera have been tested and found to be nonreactive for Hepatitis B surface antigen and HIV I. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these standard sera in the same way as potentially infectious material. Dispose patient samples and all materials used to perform this test as if they contain infectious agents.
- Do not exchange conjugates or coated microtiterstrips from kits with different lot numbers.
- Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

## STORAGE CONDITIONS



- Do not expose the **Chromogen Solution** to strong light or high temperature during storage. This solution should be colourless; if not, it should be replaced.
- Store the microtiterstrips in their original bag with the desiccant until all strips have been used.
- Never use any kit components beyond the expiration date.

## SPECIMEN COLLECTION AND PREPARATION

Human serum or plasma as well as other biological fluids may be used in this assay.

**If serum or plasma is used, it is very important to remove serum from clot as soon as possible to avoid hemolysis.** Lypemic and/or hemolyzed samples can cause erroneous results.

Separation of the serum from the clot is also important to avoid false positive results due to eventual release of TNF- $\alpha$  by the monocytes after the blood has been drawn. Small amounts of LPS or other stimuli in the collection tube may interfere with the determination if the serum is not separated from the clot.

Normal human serum or plasma does not have to be diluted. The appropriate dilution of other test samples should be determined separately. Dilutions should be made in Zero Standard/Sample Diluent, or in appropriate buffers (e.g. culture medium).

Specimens may be stored at 2-8°C for a few days, or they can be stored frozen for a longer period of time.

Avoid repeated freezing and thawing.

## ASSAY PROCEDURE

### General Remarks

- Use a separate disposable tip for each sample transfer to avoid cross-contamination.
- All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the assay has been started, all steps should be completed without interruption.

### Reconstitution of reagents.

#### Washing Buffer

Dilute 100 ml of concentrated Washing Buffer to 2000 ml with deionised water.

**CAUTION:** Crystals can be formed when the concentrated washing solution is stored at 2-8°C. These crystals can easily be dissolved when bringing the vials at room temperature or by putting them in a waterbath at 37°C.

### Standards and sample dilution

Reconstitute the content of one vial with 1 ml Zero Standard/Sample Diluent to obtain a TNF- $\alpha$  stock solution of +/- 10 ng/ml. Prepare a standard series of 10 - 30 - 100 - 300 - 1000 pg/ml by making appropriate dilutions of the stock solution with sample diluent 1 or sample diluent 2.

When testing **serum samples**, the **sample diluent 1** has to be used for the reconstitution of the standard and the preparation of the standard series. If necessary Serum samples can also be diluted in this matrix.

When testing **other biological fluids or culture supernatants**, the reconstitution of the lyophilized standard and the preparation of the standard series can be done with **sample diluent 2**. Addition of (animal) serum or proteins to this matrix can be necessary, so that it resembles the matrix of the test samples more.

Alternatively, other buffers, constituted of a matrix resembling that of the test samples, can be used to reconstitute the lyophilized standard and to prepare the standard series.

### **Assay Procedure**

1. Place the desired number of strips in the microtiterstrip holder.
2. Prepare standards as described under “**reconstitution of reagents**” and samples as described under “**specimen collection and preparation**”.
3. Pipet 125  $\mu$ l of the standards and the prepared samples into each pair of adjacent wells.
4. Incubate the covered microtiterstrips in a moist atmosphere for  $20 \pm 2$  min. at 37°C.
5. Invert the microtiterstrips over a suitable container and briskly shake out the contents. Immerse the strips immediately in the reconstituted Washing Solution. This washing step is performed 5 times. It is important to change the washing solution between the consecutive washing steps. During the third step, the washing solution is left in the strips for 2-3 min. Finally empty the microtiterstrips and remove excess fluid by blotting the inverted strips on absorbent paper. Alternatively an automatic device can be used.
6. Add 125  $\mu$ l of the Biotin-anti-TNF- $\alpha$  conjugate and incubate the covered microtiterstrips in a moist atmosphere for  $20 \pm 2$  min. at 37°C.
7. Repeat the washing cycle as described in step 5.
8. Add 125  $\mu$ l of the Streptavidin-HRP conjugate and incubate the covered microtiterstrips in a moist atmosphere for  $20 \pm 2$  min. at 37°C.
9. Repeat the washing cycle as described in step 5.
10. Add 125  $\mu$ l of Chromogen Solution to each well.
11. Incubate for  $20 \pm 2$  min. at 37°C.
12. Add 50  $\mu$ l of Stopping Solution to each well.
13. Blank the microtiterplate reader and determine the absorbance of each well at 450 nm within 30 min. following the addition of the acid.

### **RESULTS**

#### **Calculation of the Results**

Plot the average absorbance values obtained for each standard versus the corresponding TNF- $\alpha$  concentration (pg/ml) and construct a calibration curve with an optimized curve fit (e.g. log/linear).

Use the average absorbance of each patient sample and determine the patients TNF- $\alpha$  concentration by interpolation from this curve. Depending on the availability of computer capability, alternative methods of data reduction may be used.

#### **Sensitivity**

The minimum detectable concentration is expected to be less than 5 pg/ml.

### **REFERENCES**

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Human TNF- $\alpha$

Microtiterstrip ELISA kit

96 coated wells



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