

## Human TNF- $\alpha$ ELISA Kit

**[Catalog No]** EK182

**[SIZE]** 48T/96T

**[INTENDED USE]** For the quantitative determination of human Tumor Necrosis Factor alpha (TNF- $\alpha$ ) concentrations in cell culture supernates, serum and plasma.

### [INTRODUCTION]

Tumor necrosis factor alpha (TNF- $\alpha$ ), also known as cachectin and TNFSF1A, is an adipokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. It functions as a multipotent modulator of immune response and further acts as a potent pyrogen. TNF- $\alpha$  circulates throughout the body responding to stimuli (infectious agents or tissue injury), activating neutrophils, altering the properties of vascular endothelial cells, regulating metabolic activities of other tissues, as well as exhibiting tumoricidal activity by inducing localized blood clotting. TNF- $\alpha$  may play a significant role in the pathogenesis of inflammatory disease of the joints and other tissues. Recently, a growing body of information has pointed to a role for TNF- $\alpha$  in the pathogenesis of AIDS. Measurement of TNF- $\alpha$  levels has also been shown to be useful in transplant research.

### [PRINCIPLE OF THE ASSAY]

Human TNF- $\alpha$  ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of human TNF- $\alpha$  in the samples. A monoclonal antibody specific for human TNF- $\alpha$  has been immobilized onto microwells. Standard or samples are pipetted into the wells, followed by the addition of biotin-linked detect antibody specific for TNF- $\alpha$ , and TNF- $\alpha$  present is bound by the immobilized antibody and detect antibody following the first incubation. After removal of any unbound substances, streptavidin-HRP is added for a second incubation. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of TNF- $\alpha$  bound by the immobilized antibody. The color development is stopped by addition of acid and the optical density value is measured by microplate reader.

### [MATERIALS PROVIDED]

Component	Catalog Number	EK182-48	EK182-96
Coated Microplate	EK182P	48T	96T
Standard	EK182S	1vial	2vials
Detect antibody	EK182D	1vial	1vial
Standard Diluent	E0260	5ml	5ml
Streptavidin-HRP	E0290	1vial	1vial
Assay Buffer (10 $\times$ )	E0310	5ml	5ml
TMB	E0230	6ml	11ml
Stop Solution	E0300	11ml	11ml
Washing Buffer (20 $\times$ )	E0281	50ml	50ml
Adhesive Film	E0200	6	6

**Note:** Components from reagent kits of different batch numbers must not be used interchangeably.

### OTHER SUPPLIES REQUIRED

- 1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- 2) Pipettes and pipette tips.
- 3) 50  $\mu$ l to 300  $\mu$ l adjustable multichannel micropipette with disposable tips.
- 4) Multichannel micropipette reservoir.
- 5) Beakers, flasks, cylinders necessary for preparation of reagents.
- 6) Deionized or distilled water.
- 7) Polypropylene test tubes for dilution.

### [STORAGE]

Store at 2-8°C; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8°C for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20°C for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8°C for approximately 1 month.

### [SAMPLE COLLECTION AND STORAGE]

1) **Cell Culture Supernates** - Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$  for later use. Avoid repeated freeze-thaw cycles.

2) **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000  $\times$  g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$  for later use. Avoid repeated freeze-thaw cycles.

3) **Plasma** - Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000  $\times$  g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$  for later use. Avoid repeated freeze-thaw cycles.

4) Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay.

Dilution with Assay Buffer may be needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at 2 to 8°C. For longer storage, aliquot samples and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

### [REAGENT PREPARATION]

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

#### Washing Buffer (1 $\times$ )

Pour entire contents (50 ml) of the **Washing Buffer (20 $\times$ )** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25°C. Washing Buffer (1 $\times$ ) is stable for 30 days.

#### Assay Buffer (1 $\times$ )

Pour the entire contents (5 ml) of the **Assay Buffer (10 $\times$ )** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8°C. Assay Buffer (1 $\times$ ) is stable for 30 days.

#### Detect Antibody

Mix well prior to making dilutions.

Make a 1: 100 dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1 $\times$ ) in a clean plastic tube.

The diluted Detect Antibody should be used within 30 minutes after dilution.

#### Streptavidin-HRP

Mix well prior to making dilutions.

Make a 1: 100 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed.

The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

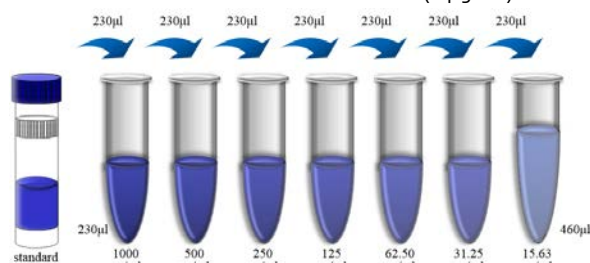
**Sample Dilution:** If your samples have high TNF- $\alpha$  content, dilute serum/plasma samples with Assay Buffer (1x). For cell culture supernates, dilute with cell culture medium.

**Human TNF- $\alpha$  Standard:** Reconstitute Human TNF- $\alpha$  Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 2,000 pg/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

- **For serum/plasma samples,** mixing concentrated human TNF- $\alpha$  standard (230  $\mu$ l) with 230  $\mu$ l of Standard Diluent creates the high standard (1,000 pg/ml). Pipette 230  $\mu$ l of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).
- **For cell culture supernates,** mixing concentrated human TNF- $\alpha$  standard (230  $\mu$ l) with 230  $\mu$ l of cell culture medium creates the high standard (1,000 pg/ml). Pipette 230  $\mu$ l of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



#### [ASSAY PROCEDURE]

Bring all reagents and samples to room temperature before use.

- 1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
- 2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
- 3) Add 300  $\mu$ l Washing Buffer (1x) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1x). Use the microwell strips immediately after washing. Do not allow wells to dry.
- 4) Add 100  $\mu$ l of 2-fold diluted Standard in duplicated. Add 100  $\mu$ l of Standard Diluent/culture medium to Blank well in duplicate.
- 5) **Serum/Plasma:** Add 50  $\mu$ l of Assay Buffer (1x) and 50  $\mu$ l sample to the sample well. **Cell culture supernates:** Add 100  $\mu$ l cell culture supernates to the sample well.
- 6) Add 50  $\mu$ l of diluted Detect Antibody to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.

7) **Seal the plate with an adhesive film.** Incubate at room temperature (25°C $\pm$ 3°C) for 2 hours on a microplate shaker set at 300 rpm.

8) Aspirate each well and wash by filling each well with 300  $\mu$ l Washing Buffer (1x), repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining Washing Buffer (1x) by aspirating or decanting. Invert the plate and tap it against clean paper towels.

9) Add 100  $\mu$ l of diluted Streptavidin-HRP to each well.

10) **Seal the plate with a fresh adhesive film.** Incubate at room temperature (25°C $\pm$ 3°C) for 45 minutes on a microplate shaker set at 300 rpm.

11) Repeat aspiration/wash as in step 8.

12) Add 100  $\mu$ l of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature (25°C $\pm$ 3°C). **Protect from light.**

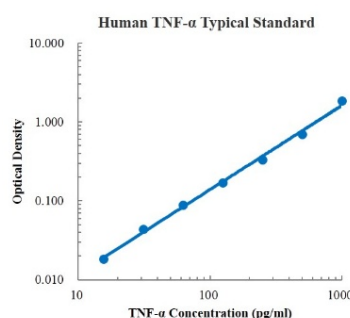
13) Add 100  $\mu$ l of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

14) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

#### [TYPICAL DATA]

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

pg/ml	O.D.	Average	Corrected
0.00	0.030	0.031	0.031
15.63	0.051	0.048	0.050
31.25	0.075	0.074	0.075
62.50	0.119	0.120	0.120
125.00	0.207	0.195	0.201
250.00	0.367	0.364	0.335
500.00	0.695	0.758	0.696
1000.00	1.850	1.907	1.879
			1.848



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