

Human TNF- α ELISA Instructions

Content

| | CAT | Volume |
|---------------------------------|------------------|--------------|
| 1 CP (Coated Plate) | EH0002CP | 96 well |
| 2 S (Standard) | EH0002S,S1-S7,S0 | 9 vial |
| 3 DA (Detect Antibody) | EH0002DA | 6 ml/bottle |
| 4 SH (Streptavidin-HRP) | ESH01 | 12 ml/bottle |
| 5 AB (Assay Buffer 1 \times) | EAB01 | 12 ml/bottle |
| 6 TS (TMB Substrate) | ETS01 | 12 ml/bottle |
| 7 SS (Stop Solution) | ESS01 | 12 ml/bottle |
| 8 WB (Wash Buffer 10 \times) | EWB01 | 50 ml/bottle |
| 9 SF (Sealer Film) | ESF01 | 6 piece |

NOTE: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

REAGENT PREPARATION

Washing Buffer (1 \times) Preparation

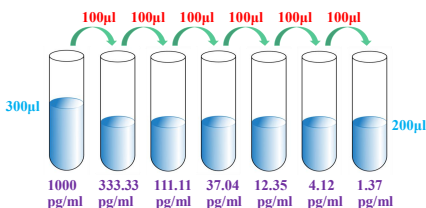
Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10 \times) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25 $^{\circ}$ C.

Standard Curve Preparation:

S1 to S7 and S0 is ready to use for serum and plasma.

Other sample type, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. Urine sample use AB (Assay Buffer) prepare standard curve.

The human TNF alpha Standard EH0002S 10000 pg/ml 30 μ l + 270 μ l SPB serves as the high standard (1000 pg/ml). Pipette 200 μ l of SPB into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/ml).

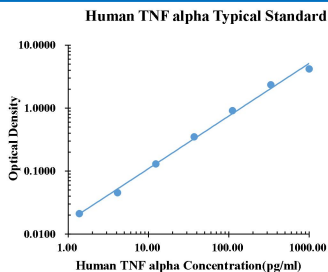


ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

- 1 Prepare all reagents and working standards as directed in the previous sections.
- 2 Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.
- 3 Add 50 μ l of **AB** (Assay Buffer) to each well.
- 4 Add 50 μ l or 10 μ l of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
- 5 Add 50 μ l of **DA** (Detect Antibody) to each well.
- 6 Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25 $^{\circ}$ C) for 1 hours on a microplate **shaker** set at 500 rpm.
- 7 Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with **WB** (Washing Buffer 300 μ l). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **WB** (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.
- 8 Add 100 μ l of **SH** (Streptavidin-HRP) to each well.
- 9 Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25 $^{\circ}$ C) for 30 min on a microplate **shaker** set at 500 rpm.
- 10 Repeat aspiration/**wash** as in step 7.
- 11 Add 100 μ l of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
- 12 Add 100 μ l of **SS** (Stop Solution) to each well.
- 13 Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA



| pg/ml | O.D. | | Average | Corrected |
|---------|--------|--------|---------|-----------|
| 0.00 | 0.0390 | 0.0402 | 0.0396 | |
| 1.37 | 0.0588 | 0.0628 | 0.0608 | 0.0212 |
| 4.12 | 0.0819 | 0.0885 | 0.0852 | 0.0456 |
| 12.35 | 0.1693 | 0.1712 | 0.1703 | 0.1307 |
| 37.04 | 0.1960 | 0.1956 | 0.3916 | 0.3520 |
| 111.11 | 0.9071 | 0.9969 | 0.9520 | 0.9124 |
| 333.33 | 2.4084 | 2.3694 | 2.3889 | 2.3493 |
| 1000.00 | 4.3651 | 4.1387 | 4.2519 | 4.2123 |

SENSITIVITY

The minimum detectable dose (MDD) of human TNF alpha is typically less than 0.21 pg/ml (50 μ l of sample volume) or 0.26 pg/ml (10 μ l of sample volume).

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

■ **Intra-assay Precision (Precision within an assay)** Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

■ **Inter-assay Precision (Precision between assays)**

| | Intra-assay Precision | | | Inter-assay Precision | | |
|------------------------------|-----------------------|------|-------|-----------------------|------|-------|
| | S1 | S2 | S3 | S1 | S2 | S3 |
| Sample Number | 22 | 22 | 22 | 6 | 6 | 6 |
| Average (pg/ml) | 13.0 | 74.8 | 215.6 | 15.3 | 73.9 | 323.3 |
| Standard deviation | 0.5 | 3.1 | 7.3 | 0.6 | 2.8 | 12.9 |
| Coefficient of variation (%) | 3.7 | 4.1 | 3.4 | 4.0 | 3.8 | 4.0 |

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human TNF alpha into health human serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 95% to 105% with an overall mean recovery of 99%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of TNF alpha in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 96% to 104% with an overall mean recovery of 100%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of TNF alpha in this assay. No medical histories were available for the donors.

| Sample Matrix | Sample Evaluated | Range (pg/ml) | Detectable % | Mean of Detectable (pg/ml) |
|---------------|------------------|---------------|--------------|----------------------------|
| Serum | 30 | 0.63-3.85 | 100 | 2.08 |

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.

CALIBRATION

The NIBSC/WHO British Standard for human leukocyte TNF alpha 88/786 was evaluated in this kit. To convert sample values obtained with the Human TNF alpha kit to relative approximate NIBSC units, use the equation below:
 NIBSC/WHO (88/786) approximate value (U/ml) = $0.058 \times$ Human TNF alpha value (pg/ml)