

Datasheet for ABIN7012842

TNF alpha ELISA Kit[Go to Product page](#)**1** Image

Overview

Quantity:	96 tests
Target:	TNF alpha
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	15.6 pg/mL - 1000 pg/mL
Minimum Detection Limit:	15.6 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a small sample sandwich enzyme immunoassay for in vitro quantitative measurement in various sample types.
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Tumor Necrosis Factor Alpha.
Sensitivity:	5.8 pg/mL
Grade:	Small Sample
Components:	<ul style="list-style-type: none">• Pre-coated, ready to use 96-well strip plate, flat bottom• Plate sealer for 96 wells

Product Details

- Reference Standard
- Standard Diluent
- Detection Reagent A
- Detection Reagent B
- Assay Diluent A
- Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- TMB Substrate
- Stop Solution
- Wash Buffer (30 x concentrate)
- Instruction manual

Target Details

Target:	TNF alpha
Alternative Name:	Tumor Necrosis Factor Alpha (TNF alpha Products)
Background:	DIF, TNF-A, TNFSF2, Cachectin, Tumor Necrosis Factor Ligand Superfamily Member 2
Pathways:	NF-kappaB Signaling , Apoptosis , Caspase Cascade in Apoptosis , TLR Signaling , Cellular Response to Molecule of Bacterial Origin , Regulation of Leukocyte Mediated Immunity , Positive Regulation of Immune Effector Process , Production of Molecular Mediator of Immune Response , Positive Regulation of Endopeptidase Activity , Hepatitis C , Protein targeting to Nucleus , Inflammasome

Application Details

Sample Volume:	25 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	<ol style="list-style-type: none">1. Prepare all reagents, samples and standards,2. Add 25µL standard or sample to each well. Incubate 1 hours at 37 °C,3. Aspirate and add 25µL prepared Detection Reagent A. Incubate 1 hour at 37 °C,4. Aspirate and wash 3 times,5. Add 25µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,6. Aspirate and wash 5 times,7. Add 25µL Substrate Solution. Incubate 10-20 minutes at 37 °C,8. Add 20µL Stop Solution. Read at 450nm immediately.
Reagent Preparation:	<ol style="list-style-type: none">1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit

will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

2. Standard - Reconstitute the Standard with 0.5 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1,000pg/mL. Prepare 7 tubes containing 0.25 mL Standard Diluent and produce a double dilution series by transferring 250 μ L each. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1,000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, 15.6pg/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0pg/mL.
3. Detection Reagent A and Detection Reagent B - If lyophilized reconstitute the Detection Reagent A with 150 μ L of Reagent Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution - Dilute 10 mL of Wash Solution concentrate (30x) with 290 mL of deionized or distilled water to prepare 300 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 μ L for once pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. Prepare Substrate working Solution within 15 minutes before assay.
6. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.

Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (\leq 1 month) or -80 °C (\leq 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.

Application Details

- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).

Assay Precision: Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate.

$CV(\%) = SD/mean \times 100$

Intra-Assay: $CV < 10\%$

Inter-Assay: $CV < 12\%$

Restrictions: For Research Use only

Handling

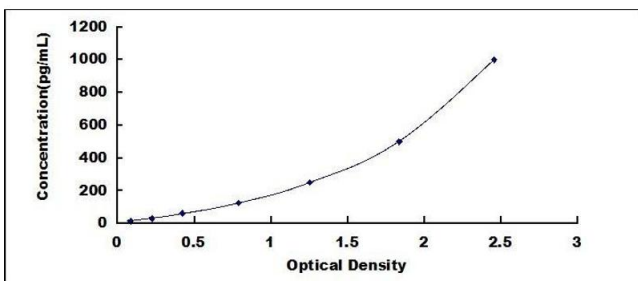
Storage: 4 °C/-20 ° C

Storage Comment: 1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent A, Detection Reagent B, and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C.

2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.

Expiry Date: 6 months

Images



ELISA

Image 1. Typical standard curve