

Datasheet for ABIN6999363

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

Overview

Quantity:	96 tests
Target:	TNF alpha
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.56 pg/mL - 100 pg/mL
Minimum Detection Limit:	1.56 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a high sensitivity 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:	0.52 pg/mL
Grade:	High Sensitivity
Components:	• 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:	100 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards, 2. Add 100µL standard or sample to each well. Incubate 1 hours at 37 °C, 3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C, 4. Aspirate and wash 3 times, 5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C, 6. Aspirate and wash 5 times, 7. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C, 8. Add 50µL Stop Solution. Read at 450nm immediately.
Reagent Preparation:	1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit is

not used up all at once, remove only the strips and reagents for the current experiment and leave the remaining strips and reagents in the desired condition.

2. **Standard** - Reconstitute the Standard with 1.0mL 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 300pg/mL. Please firstly dilute the stock solution to 100pg/mL. This diluted standard serves as the highest standard (100pg/mL). Then prepare 7 tubes containing 0.5mL of Standard Diluent and use the diluted standard to produce a double dilution series (100pg/mL, 50pg/mL, 25pg/mL, 12.5pg/mL, 6.25pg/mL, 3.12pg/mL, 1.56pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL). Mix each tube thoroughly before the next transfer.
3. **Detection Reagent A** and **Detection Reagent B** - Spin or centrifuge the stock of Detection Reagent A and B briefly before use. Dilute to working concentration (1:100) with Assay Diluent A or B, respectively.
4. **Wash Solution** - Dilute 20 mL of Wash Solution Concentrate (30x) with 580 mL of deionized or distilled water to make 600 mL of Wash Solution (1x).
5. **TMB Substrate** - Aspirate the required amount of solution with sterile tip and do not return the residual solution back into the vial.

Note:

1. Serial dilution directly in the wells is not recommended.
2. Prepare standard within 15 minutes before assay. Do not dissolve the reagents directly at 37 °C.
3. Detection Reagent A and B are sticky solutions, so pipette them slowly to reduce volume errors.
4. Reconstitute Standard or working solutions of Detection Reagent A and B carefully according to instructions, avoiding foaming and mixing gently until crystals are completely dissolved. To minimize inaccuracy caused by pipetting, use small volumes and ensure pipettes are calibrated. It is recommended to aspirate more than 10 µL for one-time pipetting.
5. The reconstituted Standard, Detection Reagent A and B can only be used once.
6. When crystals have formed in the Wash Solution concentrate (30x), warm it to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or preparation containers affect 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 (≤1 month) or -80 °C (≤ 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.

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: $CV < 12\%$
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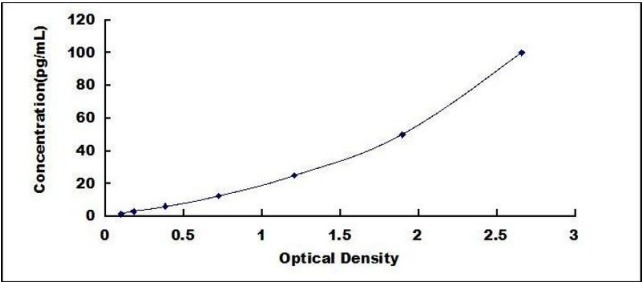
Restrictions:	For Research Use only
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Handling

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
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Images



ELISA

Image 1. Typical standard curve