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TNF alpha ELISA Kit

1	Image

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Publications



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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 sandwich enzyme immunoassay for in vitro quantitative measurement of TNFa in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates.
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 (TNFa)
Sensitivity:	6.5 pg/mL
Components:	 Pre-coated, ready to use 96-well strip plate, flat buttom Plate sealer for 96 wells 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

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Target:	TNF alpha
Alternative Name:	Tumor Necrosis Factor Alpha (TNFa) (TNF alpha Products)
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	
Comment:	Information on standard material:
	The standard might be recombinent protein or natural protein that will depend on the enecific

The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative.

Information on reagents:

The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.

Information on antibodies:

The provided antibodies and their host vary in different kits.

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
	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 1.0 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 0.5 mL Standard Diluent and produce a
	double dilution series. 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 Opg/mL.
	3. Detection Reagent A and Detection Reagent B - If lyophilized reconstitute the Detection
	Reagent A with 150µL 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 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized
	or distilled water to prepare 600 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 standards 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 10µL for one pipetting.
	4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only
	once.

and mix gently until the crystals are completely dissolved.

5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature

6. Contaminated water or container for reagent preparation will influence the detection result.

Application Details

Sample Preparation:	 It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates. If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility 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. Please estimate the concentration of the samples before performing the test. If the values are not in therange 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/meanX100 Intra-Assay: CV < 10%
Restrictions:	Inter-Assay: CV < 12% For Research Use only
Handling	
Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Storage:	4 °C/-20 °C
Storage Comment:	 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. 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

Product cited in:

Tang, Ju, Liu, Xu, Wang, Wang: "Therapeutic effect of icariin combined with stem cells on postmenopausal osteoporosis in rats." in: **Journal of bone and mineral metabolism**, Vol. 36, Issue 2, pp. 180-188, (2018) (PubMed).

Qiao, Wang, Huang, Hu, Song: "Peroxisome proliferator-activated receptor γ plays dual roles on experimental periodontitis in rats." in: **Journal of clinical periodontology**, Vol. 45, Issue 5, pp. 514-523, (2018) (PubMed).

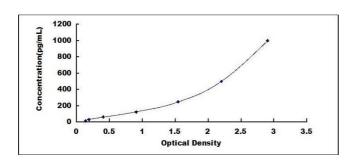
Liu, Xiong, Wang, Yang, Wang, Liu, Wu, Li, Ou, Zhang, Zhu: "Effects of water extract from epimedium on neuropeptide signaling in an ovariectomized osteoporosis rat model." in: **Journal of ethnopharmacology**, Vol. 221, pp. 126-136, (2018) (PubMed).

Gong, Yu, Zhao, Su, Sheng: "Skeletal Site-specific Effects of Zoledronate on in vivo Bone Remodeling and in vitro BMSCs Osteogenic Activity." in: **Scientific reports**, Vol. 7, pp. 36129, (2017) (PubMed).

Lama, Santoro, Corrado, Pirozzi, Paciello, Pagano, Russo, Calignano, Mattace Raso, Meli: "Extracorporeal shock waves alone or combined with raloxifene promote bone formation and suppress resorption in ovariectomized rats." in: **PLoS ONE**, Vol. 12, Issue 2, pp. e0171276, (2017) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1. Typical standard curve