KSA133Hu01 96T x5
ELISA Kit DIY Materials
For Tumor Necrosis Factor Alpha (TNFa)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

2nd Edition

[INTENDED USE]

This 'Do it Yourself (DIY)' assay kit contains materials for developing your own immunoassays. This may include the development of sandwich ELISA to measure TNFa in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids in vitro. This kit contains sufficient materials for preparation of at least five 96-well plates. Sample Preparation, Assay Protocol and Detection Range for Reference are provided as suggestions only. Researchers should optimize the use of kit materials and protocols for their own model system and determine if the kit is suitable for their samples and immunoassay.

[REAGENTS AND MATERIALS PROVIDED]

Components	Quantity	Form
Standard	1000pg	Lyophilized, 5 vials
Capture Antibody	360ug/0.3 mL	Liquid, 1 vial, contains 0.1% sodium azide
Biotinylated Detection Antibody	30ug/0.06 mL	Liquid, 1 vial, contains 0.1% sodium azide
Streptavidin-HRP	0.6 mL	Liquid, 1 vial， contains 0.05% Proclin300

TMB Substrate	48 mL	Liquid, 1 vial
96-well Plate	96 wells	5 plates

[Recommended Buffers and Solutions]

ASSAY Kit DIY Support Pack 1, **Cat # IS049**, which includes Plate Sealer, Coating Buffer, Blocking Buffer, Reagent Diluent 1, Reagent Diluent 2, Reagent Diluent 3, Wash Buffer, Enhancer is highly recommended for reagent preparation.

Notes: The recommended Cloud-Clone's diluents and buffers contained in IS049 are validated in the lab, other reagents selected for use can alter the performance of an immunoassay.

[STORAGE]

Antibodies, Standard and Streptavidin-HRP should be stored at -20°C. TMB should be stored at 4°C. 96-well Plate could be stored at room temperature. The unopened reagents are valid for 12 months, they are stable for one month after opening when stored at 4°C. Please make all solutions fresh before the experiment.

[REAGENT PREPARATION]

Bring all components to room temperature (18-25°C) before use. Working solutions should be prepared and used immediately.

Standard: Reconstitute one vial of Standard with 1.0mL of working solution of Reagent Diluent 1 (Cat # IS049), kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard is 1,000pg/mL. Then make serial dilution of the Standard with working solution of Reagent Diluent 1 in 2 times to gain a proper standard curve.

Capture Antibody: Briefly spin or centrifuge the stock Capture Antibody before use. Aspirate appropriate volume of Capture Antibody, 1: 200 dilution in working solution of Coating Buffer (Cat # IS049) for plate coating.

Detection Antibody: Briefly spin or centrifuge the stock Detection Antibody before use. Aspirate appropriate volume of Detection Antibody, 1: 1000 dilution in working solution of Reagent Diluent 2 (Cat # IS049).

Streptavidin-HRP: Briefly spin or centrifuge the stock Streptavidin-HRP before use. Aspirate appropriate volume of the reagent, 1: 100 dilution in working solution of Reagent Diluent 3 (Cat # IS049).

Cloud-Clone's product of Assay Kit DIY Support Pack 1 (Catalog: IS049), which is highly recommended for reagent preparation.

[ASSAY PROTOCOL]

Plate Preparation:

1. Dilute the Capture Antibody to working concentration in Coating Buffer (Cat # IS049). Immediately coat the 96-well microplates with 100μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at 4°C or incubate at 37°C for 2 hours.
2. Aspirate the solution and wash with 350μL of working solution of Wash Buffer (Cat # IS049) to each well using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper.
3. Block plates by adding 200μL of working solution of Blocking Buffer (Cat # IS049) to each well. Incubate at 37°C for 1.5 hours.
4. Repeat the aspiration/wash process as in Step 2. The plates are now ready for sample detection.

Commonly Used Assay Procedure:

1. Add 100μL of different concentration of standards, samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.
2. Repeat the aspiration/wash process as in Step 2 of plate preparation.
3. Add 100μL of Detection Antibody working solution to each well, cover the wells, and incubate for 1 hour at 37°C.
4. Repeat the aspiration/wash process for 3 times as in Step 2.
5. Add 100μL of working solution of Streptavidin-HRP to each well, cover the wells, and incubate for 30 minutes at 37°C.
6. Repeat the aspiration/wash process for total 5 times as in Step 2.
7. Add 90μL of TMB Substrate (Cat # IS049) to each well. Cover the wells, and incubate for 10 - 20 minutes at 37°C. Protect from light.

8.Add 50μL of Stop Solution (1mol/L H_2SO_4) to each well. Mix the liquid by tapping the side of the plate.

9.Run the microplate reader and conduct measurement at 450nm immediately.

[DETECTION RANGE FOR REFERENCE]

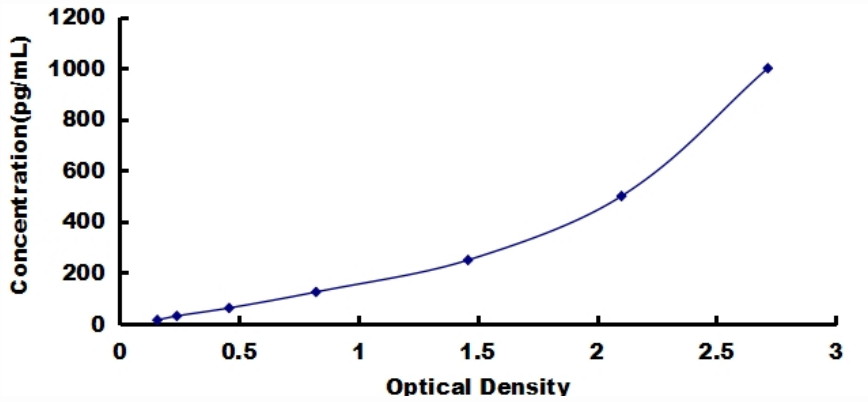
The detection range of ELISA prepared by these materials in our lab is 15.6-1,000pg/mL.

[SPECIFICITY]

The Antibody Pairs in the kit have high sensitivity and excellent specificity for detection of TNFa.

[TYPICAL DATA]

Typical standard curve below is provided for reference only. A standard curve should be generated for each experiment.



Typical Standard Curve of ELISA Assay for Human, TNFa.