DATA SHEET

SEK10602

Human TNFa (TNF-alpha / TNFSF2)

ELISA Pair Set

Background

Tumor necrosis factor alpha (TNFα), also known as Tumor necrosis factor ligand superfamily member 2, TNF-a, TNF-alpha, TNFSF2 and TNFa, is a single-pass type II membrane protein which belongs to the tumor necrosis factor family. TNFa is the prototypic cytokine of the TNF superfamily, and is a multifunctional molecule involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. TNFa is produced mainly by macrophages, and large amounts of this cytokine are released in response to lipopolysaccharide, other bacterial products, and Interleukin-1 (IL-1). TNFa is primarily produced as a type I I transmembrane protein arranged in stable homotrimers, and a soluble trimeric form of TNFa is released via proteolytic cleavage by the metalloprotease TACE/ ADAM17.

Two receptors mediating the TNFa function have been identified, the ubiquitous TNFRI from which most signaling are derived, and the hematopoietic cell-restricted TNFRII. Upon the TNFa trimers binding, TNF receptors also form trimers, undergo a conformational change, and enable the adaptor protein TRADD to bind to the death domain, and thus initiates the signal pathways such as the NF-kB, Jak/STAT, and the MAPK pathways, as well as the death signaling. As a major mediator of apoptosis, inflammation and immunity, TNFa has been implicated in the pathogenesis of a variety of human diseases including autoimmune diseases, insulin resistance, and cancer.

1. Materials provided

Capture Antibody - 0.4 mg/mL of mouse anti-TNFa monoclonal antibody. Dilute to a working concentration of 4.0 µg/mL in CBS before coating.

Detection Antibody - 0.8 mg/mL of biotinylated rabbit anti-TNFa polyclonal antibody. Dilute to a working concentration of 2.0 µg/mL in detection antibody dilution buffer before use.

Standard - Each vial contains 33ng of recombinant TNFa. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -70℃ in a manual defrost freezer. A six-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 1000 pg/mL is recommended.

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Streptavidin-HRP - 50 µL of streptavidin conjugated to horseradish-peroxidase. 1:2000 Dilution in detection antibody dilution buffer before use.

2. Sensitivity

The minimum detectable dose of human TNFa (TNF-alpha / TNFSF2) was determined to be approximately **31.25 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

3. Principle of the product

The human TNFa (TNF-alpha / TNFSF2) ELISA kit is for the quantitative determination of human TNFa.

This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for TNFa (TNF-alpha / TNFSF2) coated on a 96-well plate. Standards and samples are added to the wells, and any TNFa (TNF-alpha / TNFSF2) present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti- TNFa polyclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produces color in proportion to the amount of TNFa (TNF-alpha / TNFSF2) present in the sample strepavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450 nm.

STORAGE - Keep streptavidin-HRP at 4℃ and protect it from prolonged exposure to light. Aliquot all other reagents and store at -20℃ to -70℃ in a manual defrost freezer.

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is 3. essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.

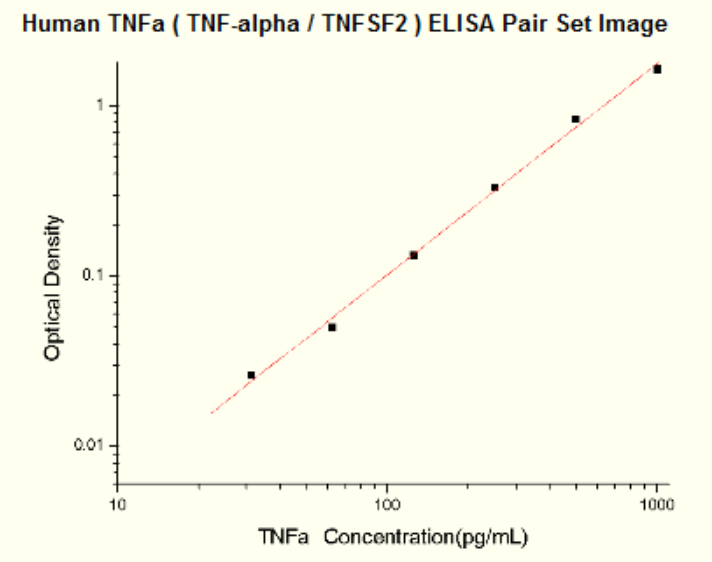
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 µL of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (if substrate solution is not as requested, the incubation time should be optimized). Avoid placing the plate in direct light.
8. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

Calculation of results

1. Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
2. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -70 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



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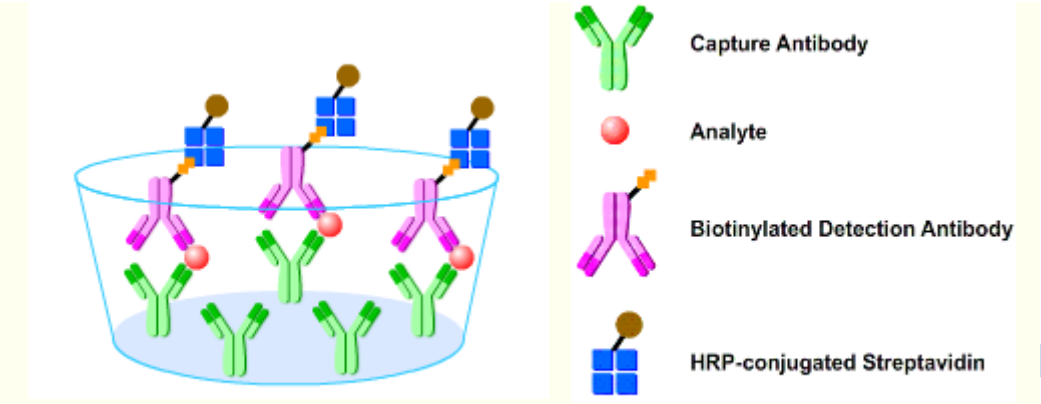
Human TNFa (TNF-alpha / TNFSF2) ELISA Pair Set Flash



This flash is a vivid summary of the assay procedure.

Related Areas:

Cancer>>Angiogenesis>>Cytokines/Chemokines in Angiogenesis>>TNF-alpha
Cancer>>Cancer Biomarkers>>TNF-alpha
Immunology>>Cytokine & Receptor>>TNF Superfamily>>TNF-alpha

Proteins:

Molecule	Species	Description //For Detailed Info. and Price------CLICK!	Cat No
TNF-alpha	Human	TNF-alpha Protein, Native **Active !**	10602-HNAE
TNF-alpha	Mouse	TNF-alpha Protein, Native **Active !**	50349-MNAE
TNF-alpha	Rat	TNF-alpha Protein, Native **Active !**	80045-RNAE
TNF-alpha	Cynomolgus	TNF-alpha Protein, Native **Active !**	90018-CNAE
TNF-alpha	Canine	TNF-alpha Protein, Native **Active !**	70003-DNAE
TNF-alpha	Ferret	TNF-alpha Protein, Native **Active !**	60002-FNAE

Antibodies:

Molecule	Application	Description //For Detailed Info. and Price------CLICK!	Cat No
Human TNF-alpha	ELISA	Mouse Monoclonal Antibody	10602-MM01
Human TNF-alpha	WB, ELISA	Rabbit Monoclonal Antibody	10602-R211
Human TNF-alpha	ELISA	Rabbit Polyclonal Antibody	10602-RP03
Mouse TNF-alpha	WB, ELISA	Rabbit Polyclonal Antibody	50349-RP01
Mouse TNF-alpha	WB, ELISA	Rabbit Polyclonal Antibody (Antigen Affinity Purified)	50349-RP02

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