Signosis
Innovative Plate Assay Solutions

Human TNFα ELISA

Catalog Number EA-0203 **(For Research Use Only)**

Introduction

Tumor Necrosis Factor alpha (TNFα), is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis. The protein is also important for angiogenesis that is critical to the growth, progression, and metastasis of solid tumors (1). Furthermore, TNFα is associated with obesity. It is chronically elevated in adipose tissues of obese rodents and humans and may represent an important link between obesity and insulin resistance (2-6). In both obese mice and humans, TNFα is overexpressed in adipose tissue. TNFα inhibits insulin signaling, at least in part by blocking insulin receptor tyrosine kinase activity and inducing serine phosphorylation of insulin receptor substrate-1 (IRS-1). However, it is unclear what the physiological stimulator of TNFα production by adipocyte during obesity is and how IRS-1 inhibits the tyrosine kinase activity of the insulin receptor after TNF-α treatment of the cells. A better understanding of the connection(s) between the TNFα and the insulin signaling pathways could be important to find a cure for the state of insulin resistance observed during obesity.

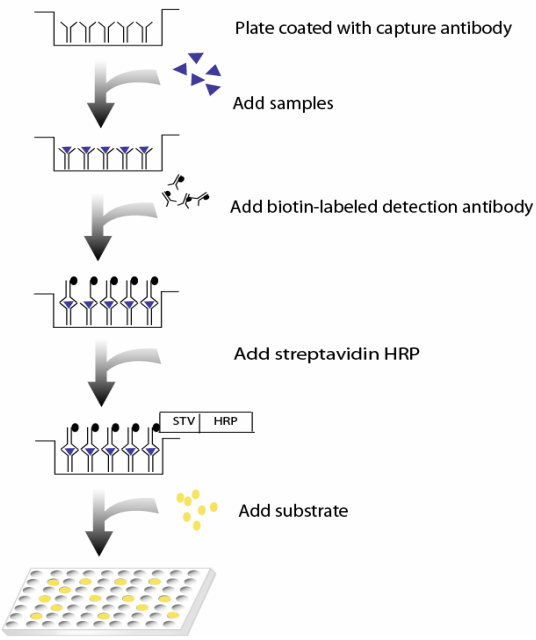

Diagram of ELISA

Principle of the assay

TNFα ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes an anti-human TNFα antibody for immobilization on the microtiter wells and a biotinylated anti-human TNFα antibody along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TNFα molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added, which results in the development of a blue color. The color development is then stopped with the addition of stop solution changing the color to yellow. The concentration of TNFα is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Materials provided with the kit

Component	Qty	Store at
8x12 96-well microplate coated with anti-human TNFα antibodies	1	4°C
Biotin-labeled goat anti-human IL-1b antibodies	25µL	-20°C
Recombinant Human TNFα standard (400ng/ml)	10µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1X Diluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5X Assay wash buffer to 1X buffer
  40ml 5X Assay wash buffer
  160ml ddH2O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- **Standard Curve Preparation**: Prepare 8,000 pg/ml Human TNFα standard by diluting 4µl of the provided Human TNFα standard (400 ng/ml) in 200µl 1X Diluent Buffer. Then, do 2-fold serial dilutions six times (Standard curve is 7 wells plus 1 blank well). Add 100µl of the diluted standards to each well.

Standard#	TNFα Concentration (pg/ml)
1	8,000
2	4,000
3	2,000
4	1,000
5	500
6	250
7	125
8	0

- Dilute biotin-labeled anti-human TNFα 1:400 with 1X Diluent buffer before use.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer before use.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100µl of sample or standard per well and incubate for 1-2 hours at room temperature (or overnight at 4°C) with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100µl of diluted biotin-labeled anti-human TNFα antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
9. Add 100µl of substrate to each well and incubate for 10-30 minutes.
10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Immediately measure the plate with a microplate reader at 450 nm.