Signosis
Innovative Plate Assay Solutions

Mouse TNFα ELISA

Catalog Number EA-2203 **(For Research Use Only)**

Introduction

Tumour 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) (7). However, it is unclear what the physiological stimulator of TNF-α 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 TNF-α and the insulin signaling pathways could be important to find a cure for the state of insulin resistance observed during obesity.

Principle of the assay

TNF-α ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes goat anti-mouse TNF-α antibodies for immobilization on the microtiter wells and goat anti-mouse TNF-α antibodies 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 TNF-α 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 to result 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 TNF-α is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

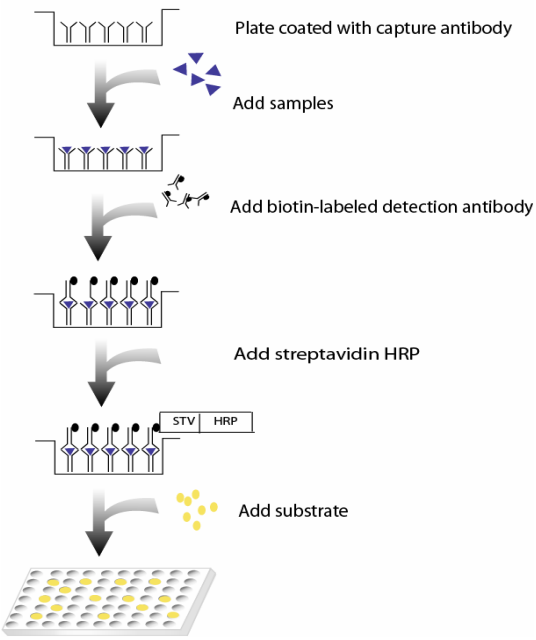


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-Well 12 strip Plate coated with goat anti-mouse TNFα antibodies	1	4°C
Biotin labeled anti-mouse TNFα antibody	25µL	-20°C
Recombinant mouse TNF-α 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

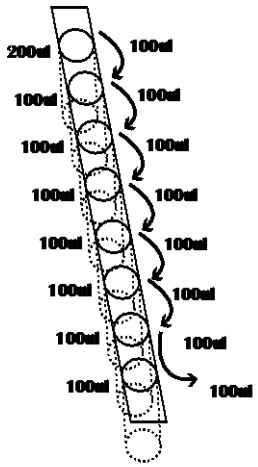
- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of mouse TNFα recombinant protein (400ng/ml) with 1X Diluent buffer to 8ng/ml by adding 4µl Mouse TNFα recombinant protein in first well with 200µl Diluent buffer then 2-fold serial dilutions (see step 2 in Assay procedure below for the detailed instruction).
- Dilute 400 times of biotin labeled anti-mouse TNFα antibody with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.
- 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.**

Sample preparation before starting experiment

- For **media samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X Diluent buffer, for example, add 10ul sample in 90ul 1X Diluent buffer.

Assay procedure

1. Take the desired the number of samples to decide how many strips need to be used. Make sure the rest of strips are well sealed.
2. Standard curve:



a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100µl of sample to each well, incubate for 1-2 hours at room temperature with gentle shaking
4. 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.
5. Add 100 µl of diluted biotin-labeled anti-mouse TNFα antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. 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.**
10. Add 100µl of substrate to each well and incubate for 5-30 minutes.
11. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
12. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.