Signosis
Innovative Plate Assay Solutions

Rat TNFα ELISA

Catalog Number EA-3001 **(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 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.

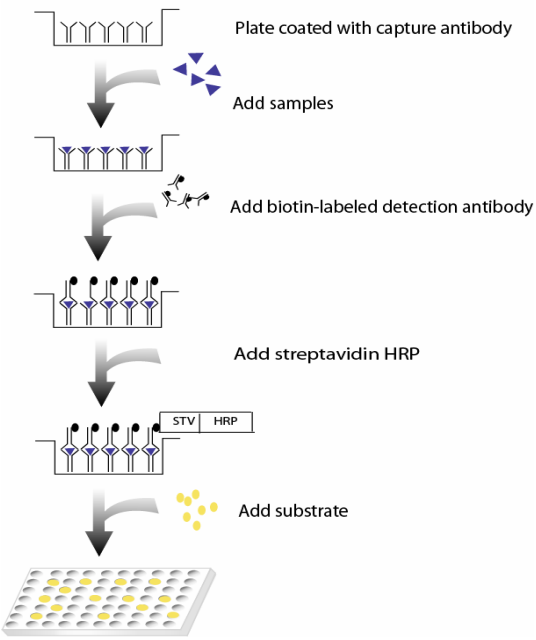


Diagram of ELISA

Principle of the assay

TNF-α ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes goat anti-rat TNF-α antibodies for immobilization on the microtiter wells and goat anti-rat 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.

Materials provided with the kit

- 96 well microplate coated with goat anti-rat TNFα antibodies (4°C).
- Biotin labeled goat anti-rat TNFα antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Rat recombinant TNF-α standard (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer ((4°C)
- Substrate (4°C).
- Stop Solution (4°C).

Material required but not provided

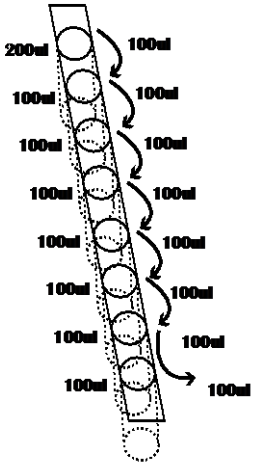
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

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 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 100 times of Rat recombinant TNFα (200ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions.
  Add 2ul Rat Recombinant TNFα in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled goat anti-rat TNF-α antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. See instruction and diagram below for standard preparation.



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 100ul of sample per well and incubate for 1 hour 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. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100µl of diluted biotin-labeled anti-rat TNFα 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. 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. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example of standard curve