



## Mouse TNF $\alpha$ ELISA

Catalog Number EA-2203

(For Research Use Only)

### Introduction

Tumour Necrosis Factor alpha (TNF $\alpha$ ), 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, TNF $\alpha$  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, TNF $\alpha$  is overexpressed in adipose tissue. TNF $\alpha$  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- $\alpha$  production by adipocyte during obesity is and how IRS-1 inhibits the tyrosine kinase activity of the insulin receptor after TNF- $\alpha$  treatment of the cells. A better understanding of the connection(s) between the TNF- $\alpha$  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- $\alpha$  ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes goat anti-mouse TNF- $\alpha$  antibodies for immobilization on the microtiter wells and goat anti-mouse TNF- $\alpha$  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- $\alpha$  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- $\alpha$  is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

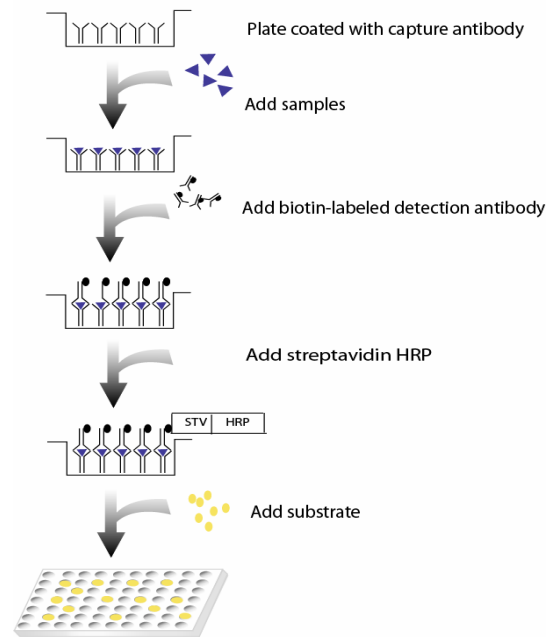


Diagram of ELISA

### Materials provided with the kit

| Component  | Qty        | Store at |
|--|------------|----------|
| <b>8x12 96-Well 12 strip Plate coated with goat anti-mouse TNF<math>\alpha</math> antibodies</b> | 1          | 4°C      |
| Biotin labeled goat anti-mouse TNF $\alpha$ antibodies   | 25 $\mu$ L | -20°C    |
| Recombinant mouse TNF- $\alpha$ standard (400ng/ml)  | 10 $\mu$ L | -20°C    |
| <b>Streptavidin-HRP conjugate</b>  | 50 $\mu$ L | 4°C      |
| <b>1xDiluent buffer</b>  | 40mL       | 4°C      |
| <b>5X Assay wash buffer</b>  | 40mL       | 4°C      |
| <b>Substrate</b>   | 10mL       | 4°C      |
| <b>Stop solution</b>   | 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 ddH<sub>2</sub>O
- Dilute 100 times of mouse TNF $\alpha$  recombinant protein (400ng/ml) with 1X Diluent buffer to 4ng/ml by adding 2 $\mu$ l Mouse TNF $\alpha$  recombinant protein in first well with 200 $\mu$ l diluent buffer then 2-fold serial dilutions (see step 2 in Assay procedure below for the detailed instruction).
- Dilute 400 times of biotin labeled mouse anti TNF $\alpha$  antibody with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

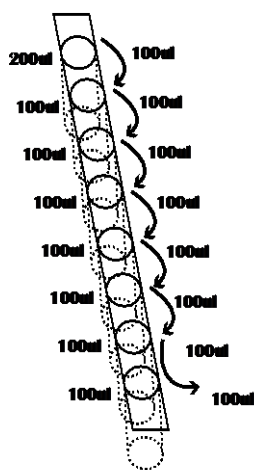
## Sample preparation before starting experiment

- For **media samples**, add 100 $\mu$ l 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 1<sup>st</sup> 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 1<sup>st</sup> well and transfer 100ul from the 1<sup>st</sup> well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100 $\mu$ l of sample to each well, incubate for 1-2 hours at room temperature with gentle shaking

4. Aspirate each well and wash by adding 200 $\mu$ l 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  $\mu$ l of diluted biotin-labeled anti-mouse TNF $\alpha$  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  $\mu$ l 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 100 $\mu$ l of substrate to each well and incubate for 5-30 minutes.
10. Add 50 $\mu$ l 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.