Signosis
Innovative Plate Assay Solutions

Mouse IL-6 LISA

Catalog Number EA-2206 **(For Research Use Only)**

Introduction

Interleukin-6 (IL-6) is a multifunctional cytokine that regulates the immune response, hematopoiesis, the acute phase response, and inflammation. Deregulation of IL-6 production is implicated in the pathology of several disease processes. Its levels are observed in several diseases, including rheumatoid arthritis (RA). IL-6 plays roles in both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response. In addition, like TNFα, IL-6 is another adipocyte secretory product that may be involved in insulin resistance. IL-6 is a cytokine secreted by many cells, including adipocytes and adipose stromal cells. IL-6 secretion is increased in the adipocytes of obese subjects (1) and may be important either as a circulating hormone or as a local regulator of insulin action (2-4). IL-6 has been implicated in the development of insulin resistance and type 2 diabetes in obese individuals. Like TNF, IL-6 inhibits the expression of LPL, but, unlike TNF, IL-6 does not stimulate lipolysis (5, 6).

Principle of the assay

IL-6 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-mouse IL-6 antibody for immobilization on the microtiter wells and rabbit anti-mouse IL-6 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 IL-6 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 IL-6 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

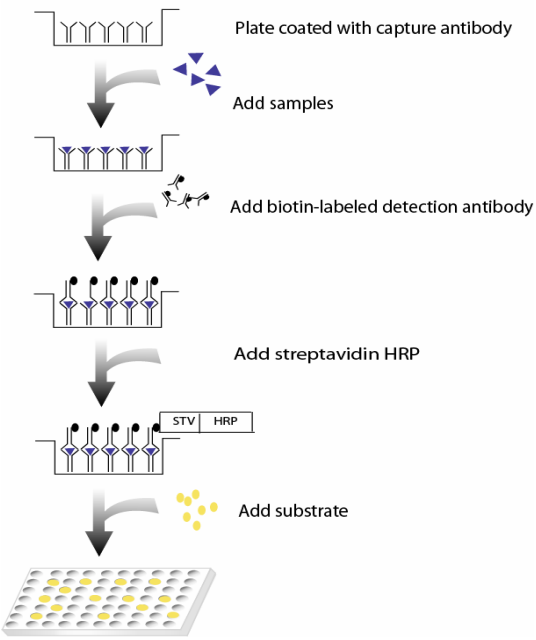


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with rabbit anti-mouse IL-6 antibodies (4°C).
- Biotin labeled rabbit anti-mouse IL-6 antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Mouse recombinant IL-6 standard (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (4°C).
- Substrate (4°C).
- Stop Solution (RT).

Material required but not provided

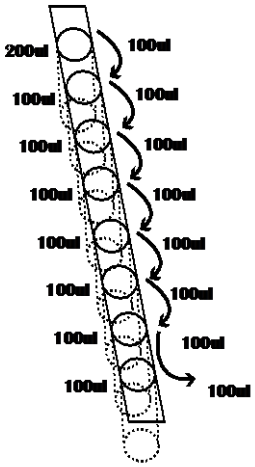
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

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 Mouse recombinant IL-6 (200ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. To dilute 100 times of Mouse recombinant IL-6, add 2ul Mouse Recombinant IL-6 in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled rabbit anti-mouse IL-6 antibody 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-mouse IL-6 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. 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.

References

(1) Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, and Coppack SW. 1997 Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 82: 4196–4200.
(2) Bastard JP, Jardel C, Bruckert E, et al. 2000. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weitht loss. J. Clin. Endocrinol Metab 85:3338-3342.
(3) Kern, PA, Ranganaan S, Li C, et al. 2001, Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance 2. Am. J. Physiol – Endocrinal metab. 280:E745-751.

Example of standard curve