Signosis
Innovative Plate Assay Solutions

Human IL-6 ELISA

Catalog Number EA-0206 **(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.

Principle of the assay

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

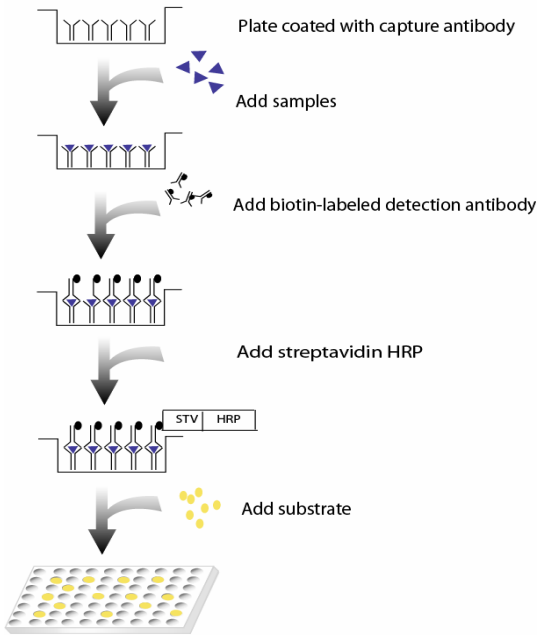

Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-well microplate coated with anti-human IL-6 antibodies	1	4°C
Biotin-labeled goat anti-human IL-1b antibodies	25µL	-20°C
Recombinant Human IL-6 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 IL-6 standard by diluting 4µl of the provided Human IL-6 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#	IL-6 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 IL-6 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 IL-6 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.