Signosis
Innovative Plate Assay Solutions

Human Obesity ELISA Strip for Profiling 8 Cytokines

Catalog Number EA-1001 **(For Research Use Only)**

Introduction

Obesity increases the risk for the metabolic syndrome, diabetes, hypertension, atherosclerosis, and thrombosis (1-5). A number of proteins have been identified to be relevant to the development of the metabolic syndrome, diabetes, and cardiovascular disease with obesity. These include adiponectin, leptin, TNFα, IGF-1, Resistin, TGFβ, IL-6, and PAI-1. Plasma concentrations of these proteins are usually measured by ELISA. To systematically examine the effects, Signosis developed an ELISA Strip Profiling Assay simultaneously profile 8 proteins; TNFa, IGF-1, Resistin, IL-6, PAI-1, TGFb, adiponectin and leptin. The difference of these proteins between two samples can be determined through data comparison. Therefore, it facilitates the discovery of the change of these proteins in different samples.

Principle of the assay

In each well of the strip, a primary antibody against a specific obesity cytokine is coated and 8 wells of the strip are coated with 8 different antibodies. Therefore, total 8 wells of a strip allow measurement of 8 different cytokines. The test sample is allowed to react simultaneously with pairs of two antibodies, resulting in the obesity cytokines 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 concentrations of the obesity cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Materials provided with the kit

Component	Qty	Store at
12 strips, each coated with 8 different antibodies against human obesity cytokines	1	4°C
Biotin labeled antibody mixture against 8 different human obesity cytokines	200µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	RT

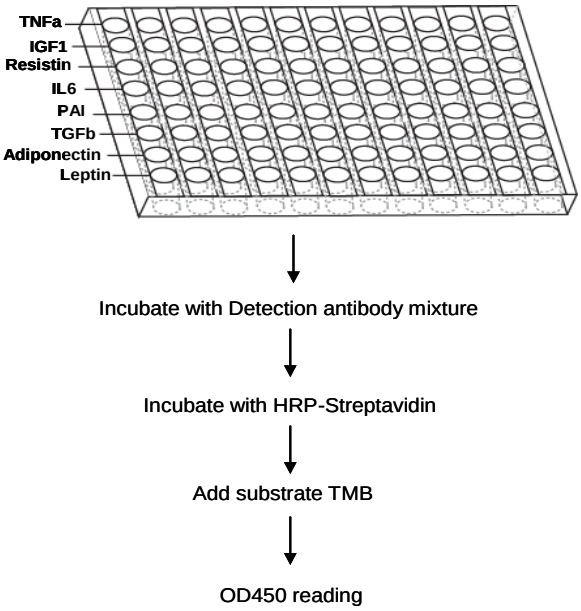


Diagram of Human Obesity ELISA Strip Analysis

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40ml 5x Assay wash buffer
  - 160ml ddH2O
- Refer to Standards User Manuel for diluting standards.
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100ul directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol on Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Recommendation

- The product intends to be used for comparison of 12 different samples. The differences of the obesity markers among the samples can be easily identified and determined.
- If you would like to quantitatively measure the cytokines in the samples, please order EA-1002. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Following EA-1002 user manual)

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of wells are well sealed.
2. Standard curve:
If protein standard curve is desired, 4-5 strips may be used to make Standard curve (Please see the user manual for EA-1002 for detail).
3. Sample assay:
Apply each sample in one strip, 100µl per well and incubate for 1-2 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. Completely remove 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 antibody mixture 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 substrate to each well and incubate for 10-30 minutes.

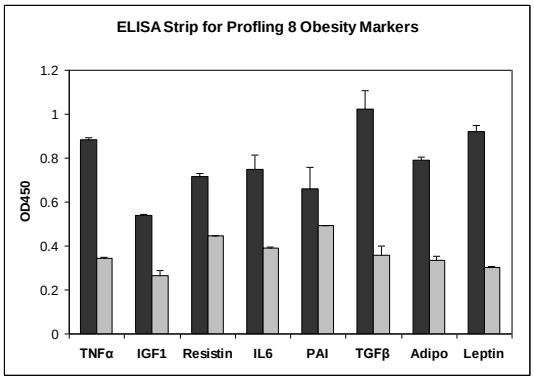
Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes. Always stop the reaction of samples from the same row at the same time.

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. Moller DE, Flier JS. Insulin resistance—mechanisms, syndromes, and implications. N Engl J Med 1991;325:938–48.
2. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983;67:968–77.
3. Loskutoff DJ, Samad F. The adipocyte and hemostatic balance in obesity. Studies of PAI-1. Arterioscler Thromb Vasc Biol 1998;18: 1–6.
4. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
5. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 2002;287:356–9.

Example of standard curve



	Black bar	Grey bar
TNFα	290pg/ml	58pg/ml
IGF1	220pg/ml	44pg/ml
Resistin	300pg/ml	60pg/ml
IL6	35pg/ml	7pg/ml
PAI	130pg/ml	26pg/ml
TGFβ	280pg/ml	56pg/ml
Adipo	750pg/ml	150pg/ml
Leptin	10ng/ml	2ng/ml