Signosis
Innovative Plate Assay Solutions

Rat Obesity ELISA Strip for Profiling 8 Cytokines

Catalog Number EA-1221 **(For Research Use Only)**

Introduction

Obesity increases the risk for the metabolic syndrome, diabetes, hypertension, atherosclerosis, and thrombosis. A number of proteins have been identified to be relevant to the development of the metabolic syndrome, diabetes, and cardiovascular disease with obesity. Plasma concentrations of these proteins are usually measured by ELISA. To systematically examine the effects, Signosis developed a Rat Obesity ELISA Strip Profiling Assay which allows simultaneous determination of 8 proteins; TNFa, IL-6, IFNr, IL-1a, IL-1b, MCP-1, VEGF, 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 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 rat obesity cytokines	1	4°C
Biotin labeled antibody mixture against 8 different rat 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

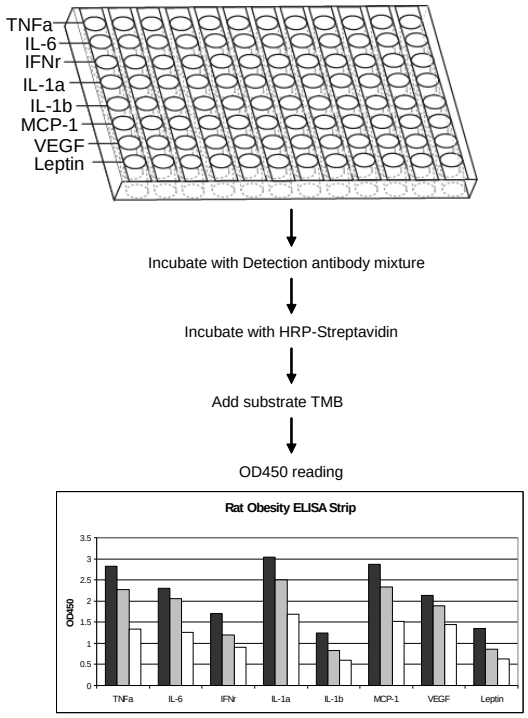


Diagram of Rat Obesity ELISA Strip Analysis

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40ml 5x Assay wash buffer
  - 160ml ddH2O
- 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 proteins in the samples, please order EA-1222. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Following EA-1222 user manual)

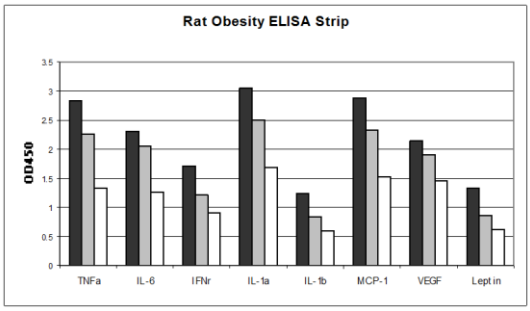
Assay procedure

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-1222 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.

Example of standard curve



	Black bar	Grey bar	White
Rat TNFa	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat IL-6	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat IFNr	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat IL-1a	1.25ng/ml	0.63ng/ml	0.31ng/ml
Rat IL-1b	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat MCP-1	1.25ng/ml	0.63ng/ml	0.31ng/ml
Rat VEGF	0.625ng/m	0.313ng/m	0.156ng/ml
Rat leptin	2.5ng/ml	1.25ng/ml	0.625ng/ml