Rat Obesity ELISA Kit

Cat.No: DEIABL71
Lot. No. (See product label)

Size

96T

Intended use

Rat Obesity ELISA Kit allows simultaneous determination of 8 proteins concentrations: TNFa, IL-6, IFNr, IL1a, IL1b, MCP-1, VEGF and Leptin in the plasma. For Research Use Only

General Description

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, CD developed an ELISA strip which allows simultaneous determination of 8 proteins: TNFa, IL-6, IFNr, IL1a, IL1b, 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 Test

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.

Reagents And Materials Provided

1. 12 strips, each coated with 8 different antibodies against rat obesity cytokines (4°C)
2. Biotin labeled antibody mixture against 8 different obesity cytokines (-20°C)
3. Streptavidin-HRP conjugate (4°C)
4. 1x Diluent buffer (4°C)
5. 5x Assay wash buffer (4°C)
6. Substrate (4°C)
7. Stop Solution (4°C)

Materials Required But Not Supplied

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1. Dilute the 5x Assay wash buffer to 1x buffer 40 ml 5x Assay wash buffer 160 ml ddH2O
2. Use original or 10-fold diluted serum-free conditioned media, cell lysates, or sera. Samples can be diluted with 1x Diluent buffer.
3. Dilute 50 times of biotin labeled antibody mixture with 1x Diluent buffer.
4. Dilute 200 times of streptavidin-HRP with 1x Diluent buffer.

Storage

4°C, but Biotin labeled antibody mixture against 8 different obesity cytokines (-20°C)

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. Add 100 µL of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200 µL 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.
4. Add 100 µL of diluted biotin-labeled antibody mixture 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. Add 100 µL substrate to each well and incubate for 10-30 minutes depending on signal intensity. Shake gently for best results.

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

9. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

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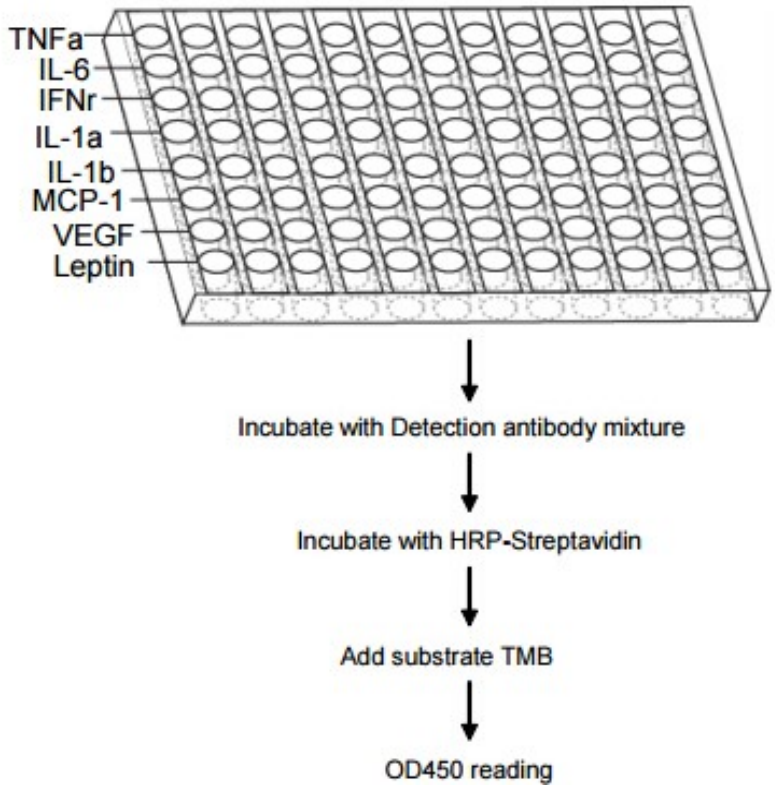
Diagram of Rat Obesity ELISA Kit Analysis:



Typical Standard Curve

Example of standard curve:

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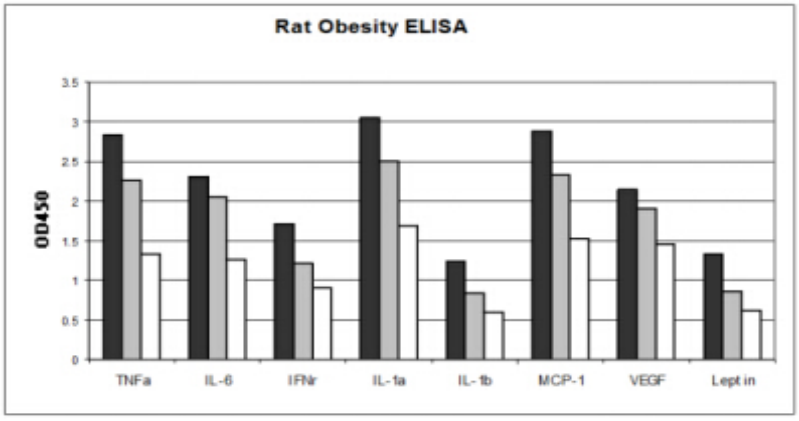

	Black bar	Grey bar	White
Rat TNFa	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat IL-6	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat IFNr	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat IL-1a	1.25ng/ml	0.63ng/ml	0.31ng/ml
Rat IL-1b	5.0ng/ml	2.5ng/ml	1.25ng/ml
Rat MCP-1	1.25ng/ml	0.63ng/ml	0.31ng/ml
Rat VEGF	0.625ng/m	0.313ng/m	0.156ng/ml
Rat leptin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

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