Signosis
Innovative Plate Assay Solutions

Mouse Resistin ELISA

Catalog Number EA-220X **(For Research Use Only)**

Introduction

Adipocytokines are a group of diverse effector molecules that are produced by adipose cells. Abnormal expression of adipokines such as Resistin is associated with the development of insulin resistance, diabetes and other metabolic and cardiovascular disorders in man. Mouse Resistin was described as a novel obesity-mediated adipocytokine that impairs glucose homeostasis by affecting both insulin-stimulated glucose uptake in adipose tissue and hepatic glucose production during fasting. However, there were initially two opposite views on human Resistin. Some studies have shown the ccorrelations between Resistin and obesity. Serum Resistin levels increase with increased adiposity and conversely, decline with decreased adiposity following medical treatment. However, other studies presented contradictory evidences that significantly decreased serum concentrations of resistin with increased adiposity. Resistin has also been shown to increase the expression of several pro-inflammatory cytokines including IL-1, IL-6, IL-12, and TNF-α via NF-κB.

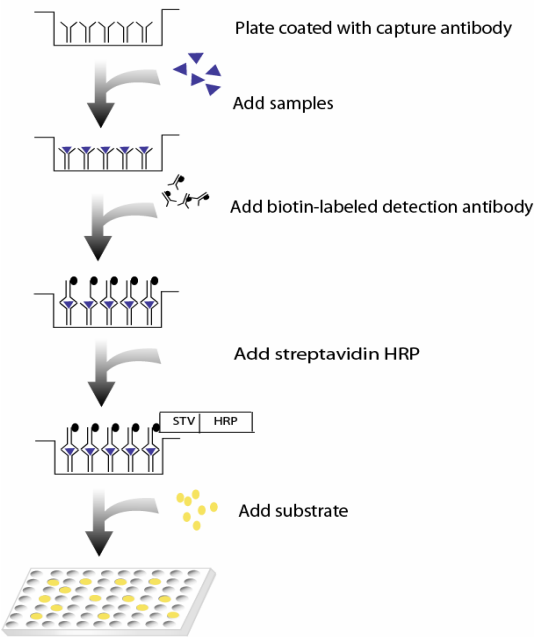


Diagram of ELISA

Principle of the assay

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

Materials provided with the kit

- 8x12 96-well microplate coated with rabbit anti-mouse resistin antibody (4°C).
- Biotin labeled rabbit anti-mouse resistin antibody (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant mouse resistin standard (40ng/ml) (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

Material required but not provided

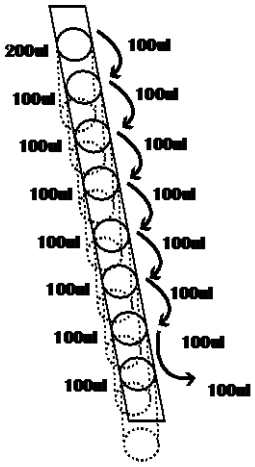
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

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 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 resistin (40ng/ml) with 1X Diluent buffer to 400pg/ml and then 2-fold serial dilutions.
- Dilute 400 times of biotin labeled rabbit anti-mouse resistin antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100 µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.



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. 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.
4. Add 100 µl of diluted biotin-labeled rabbit anti-mouse leptin antibodies 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 100µl of substrate to each well and incubate for 5-30 minutes.
9. Add 50µl 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.

Example of standard curve