Signosis
Innovative Plate Assay Solutions

Mouse IGF-I ELISA

Catalog Number EA-2204 **(For Research Use Only)**

Introduction

Insulin-like growth factor-I (IGF-I) acts as an important mediator between growth hormone and growth throughout fetal and childhood development. More circumstantial evidence indicates the association of IGF-I to the risk of cancer. High concentrations of IGF-I has been shown to be an increased risk of colorectal cancer and breast cancer in some studies and less consistently with prostate, thyroid, and haematological malignancies (1). IGF-I is a potent mitogen and important stimulus for adipocyte differentiation. IGF-I can reduce hyperglycemia in patients with severe insulin resistance by direct effects mediated via the IGF-I receptor (2). IGF-I infusion lowers insulin and lipid levels in healthy humans and reduces plasma leptin concentrations in rats (3), suggesting that IGF-I may reduce the degree of insulin resistance in type 2 diabetes, obesity, and hyperlipidemia (4).

Principle of the assay

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

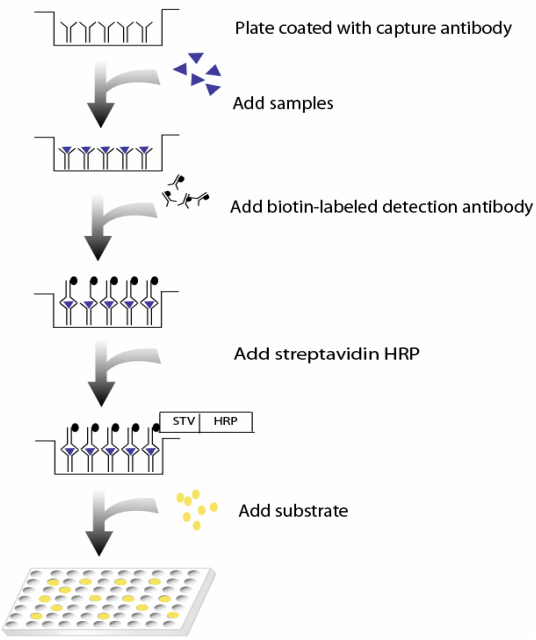

Diagram of ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with goat anti-mouse IGF-I antibodies (4ºC).
- Biotin labeled goat anti-mouse IGF-I antibodies (-20ºC).
- Streptavidin-HRP conjugate (4ºC).
- Mouse recombinant IGF-I standard (-20ºC).
- 1X Diluent buffer (4ºC).
- 5X Assay wash buffer (4ºC)
- Substrate (4ºC).
- Stop Solution (4ºC).

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
  40 ml 5x Assay wash buffer
  160 ml 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 50 times of Mouse recombinant IGF-1 (200 ng/ml) with 1X Diluent blocking buffer to 4000 pg/ml and then 2-fold serial dilutions. Add 4 ul Mouse recombinant IGF-1 in 200 ul 1X Diluent Buffer (See Step 2 below for detailed instruction).
- Dilute 400 times of biotin labeled goat anti-mouse IGF-I antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100 ul directly to the well or dilute 2-fold with 1X Diluent buffer.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol on Cell Lysate Buffer User Manual on our website.
- For **serum or plasma samples**, we recommend a 1:10 to 1:20 dilution with 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.
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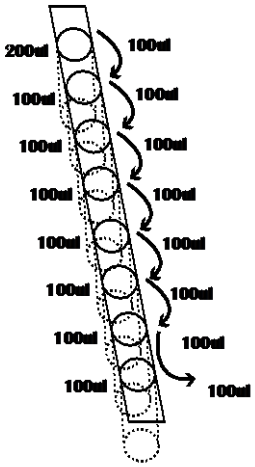
Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. See instruction and diagram below for standard preparation.



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. Add 100 ul of sample per well and incubate for 1 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. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100 µl of diluted biotin-labeled mouse anti-human Resistin antibody 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 100 µl of substrate to each well and incubate for 5-10 minutes.
10. Add 50 µl 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.