Signosis
BioSignal Capture

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.

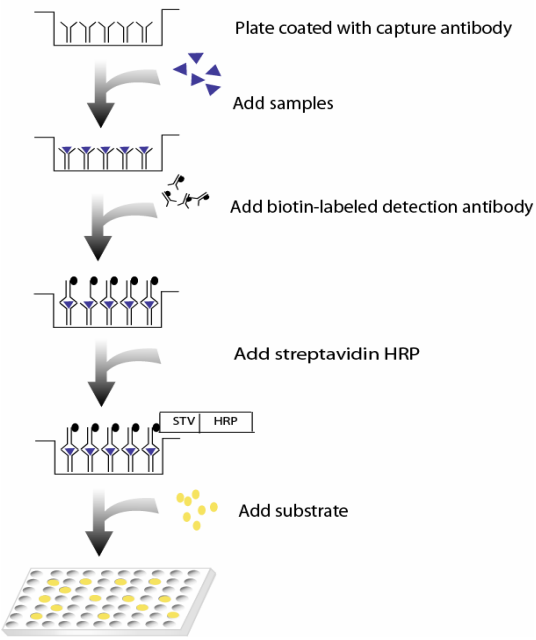


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with goat anti-mouse IGF-I antibodies (4°C).
- Biotin labeled goat anti-mouse IGF-I antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Mouse recombinant IGF-I standard (200ng/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

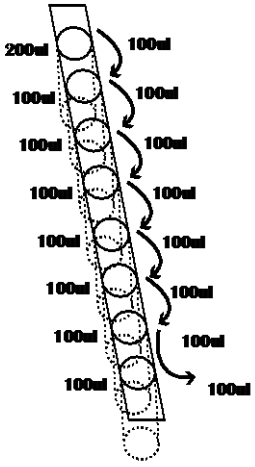
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 50 times of Mouse recombinant IGF-1 (200ng/ml) with 1X Diluent blocking buffer to 4000pg/ml and then 2-fold serial dilutions. Add 4ul Mouse recombinant IGF-1 in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction).
- Dilute 200 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.

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. 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 100ul 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 100µl 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 100µl of substrate to each well and incubate for 5-10 minutes.
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) Jenkins P. Cancer in acromegaly. Trends Endocrinology Metab 1998; 9: 360-366.
(2) Dunger DB, Acerini CL 1997 Does recombinant human insulin-like growth factor-1 have a role in the treatment of diabetes? Diabet Med 14:723–731.
(3) Boni-Schnetzler M, Hauri C, Zapf J 1999 Leptin is suppressed during infusion of recombinant human insulin-like growth factor I (rhIGF I) in normal rats. Diabetologia 42:160–166.
(4) Zenobi PD, Jaeggi Groisman SE, Riesen WF, Roder ME, Froesch ER 1992 Insulin-like growth factor-I improves glucose and lipid metabolism in type 2 diabetes mellitus. J Clin Invest 90:2234–2241.

Example of standard curve