Signosis
Innovative Plate Assay Solutions

Human Insulin ELISA

Catalog Number EA-0515 **(For Research Use Only)**

Introduction

Insulin is a vital hormone secreted by the pancreas. It is best known for its ability to control glucose levels in the blood stream; however it can also affect cellular activities such as DNA replication; protein and lipid synthesis; and the uptake of glucose, amino acids, and potassium. The severity of diseases related to insulin disruption, such as Diabetes mellitus, demonstrates just how important this molecule is to the body. The ability to simultaneously and quantifiably compare insulin level in many samples can improve our understanding of a multitude of cellular processes and disorders.

Principle of the assay

Insulin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse anti-human insulin antibody for immobilization on the microtiter wells and a biotinated mouse anti-human insulin antibody 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 insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound antibodies. A HRP substrate is added to develop a blue color. The color development is then stopped with the addition of stop solution changing the color to yellow. The concentration of insulin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

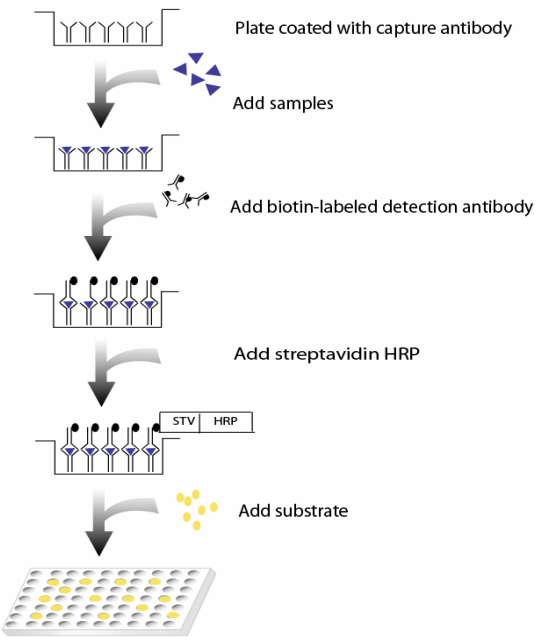


Diagram of ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with a mouse anti-human insulin antibody (4°C).
- Biotin labeled mouse anti-human insulin antibody (-20°C).
- Streptavidin-HRP conjugate (4°C)
- Human recombinant insulin standard 2 ng/ml (-20°C).
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate (4°C)
- Stop Solution (RT)

Material required but not provided

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

Reagent preparation before starting experiment

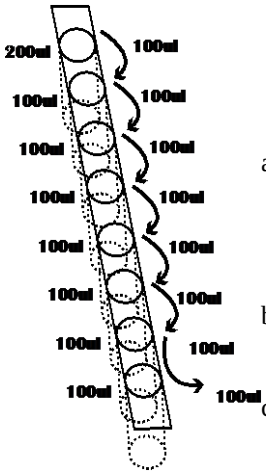
- Dilute the 5x Assay wash buffer to 1x buffer:
  40ml 5x Assay wash buffer
  160ml ddH2O.
- Dilute 100 times of Human recombinant Insulin (600ng/ml) with 1X Diluent buffer to 6000pg/ml and then 2-fold serial dilutions. Add 2ul Human Recombinant Insulin in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute biotin-labeled antibody mixture 1:400 with 1X Diluent Buffer.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer.

Sample preparation before starting experiment

- For **media samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add standard according to the diagram below or 100ul of sample per well and incubate for 1 hour at room temperature with gentle shaking



a. Add 200 µl 1X Diluent buffer to the 1st well. Add 100 µl 1X Diluent Buffer to the rest wells of the strip.
b. Add appropriate amount of recombinant insulin to the 1st well.
c. Mix the 1st well and transfer 100 µl from the 1st well to the next well.
d. Repeat procedure for each well of the strip (See picture)

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. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100µl of diluted biotin-labeled mouse anti-human insulin antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 4.
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 4.
8. Add 100µl of substrate to each well and incubate for 10-30 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.