Signosis
Innovative Plate Assay Solutions

Mouse Insulin ELISA

Catalog Number EA-2522 **(For Research Use Only)**

Introduction

Insulin is one of the major regulatory hormones of intermediate metabolism throughout the body. The biological actions of this hormone involve integration of carbohydrate, protein, and lipid metabolism. Insulin enhances membrane transport of glucose, amino acids, and certain ions. It also promotes glycogen storage, formation of triglycerides and synthesis of proteins and nucleic acids. Deficiency of insulin results in diabetes mellitus, one of the leading causes of morbidity and mortality in the general population. Insulin is also present in tumors of B cell origin such as insulinoma.

Principle of the assay

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

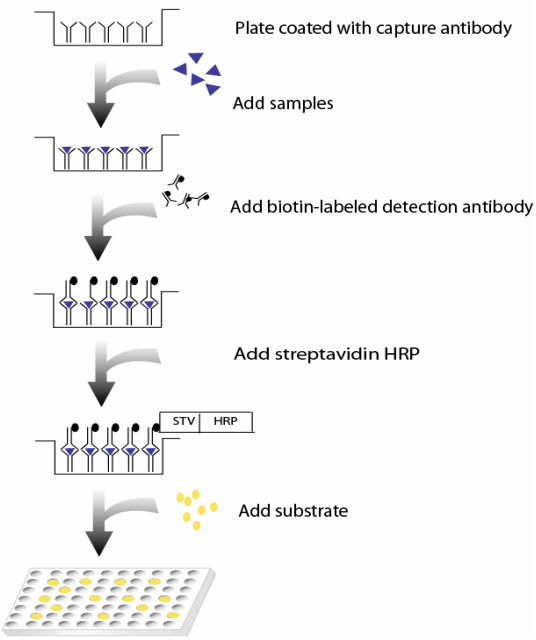


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-Well 12 strip Plate coated with mouse anti-mouse Insulin antibodies	1	4°C
Biotin labeled mouse anti-mouse Insulin antibodies	25 µL	-20°C
Recombinant mouse Insulin standard (100 ng/ml)	10 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1xDiluent buffer	40 mL	4°C
5X Assay wash buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop solution	5 mL	4°C

Material required but not provided

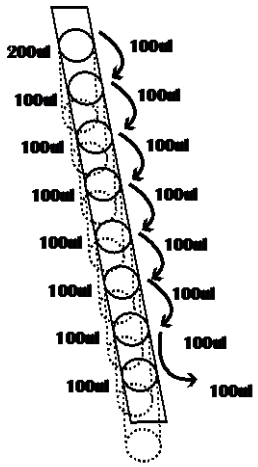
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of mouse recombinant Insulin (100 ng/ml) with 1X Diluent buffer to 1000 pg/ml and then 2-fold serial dilutions. Add 2 ul mouse recombinant Insulin in 200 ul 1X Diluent Buffer (See Step 2 in "Assay Procedure" for detailed instruction)
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control..
- Dilute 400 times of biotin labeled rabbit anti-mouse Insulin 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. Make sure the rest strips 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. 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. 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 100 µl of diluted biotin-labeled rabbit anti-mouse Insulin 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
7. Repeat the aspiration/wash as in step 3.
8. Add 100 µl substrate to each well and incubate for 5-30 minutes.
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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