Signosis, Inc.
Innovative Plate Assay Solutions

Insulin ELISA Kit

Catalog # EA-7080 **(For Research Use Only)**

Introduction

Insulin is a vital hormone responsible for regulating blood glucose levels and ensuring that cells receive the energy they need to function. This hormone plays a central role in maintaining metabolic balance and energy homeostasis. However, when insulin production or function is impaired—as seen in conditions like diabetes—blood sugar regulation is disrupted, leading to serious metabolic complications. Understanding and researching insulin is therefore essential for advancing treatments for diabetes and other metabolic disorders.

Principle

The hormone insulin is analyzed using a competitive ELISA assay. In this assay, the plate is coated with the target hormone conjugated to a protein, which competes with the free hormones in the samples that are added to the plate. After the samples containing free hormone are added to the plate, an anti-hormone antibody labeled with biotin is added to the samples and binds to both the free hormones and conjugated hormones immobilized on the plate. The plate is then washed to remove any sample and antibodies not binding to the hormones bound on the plate. The antibodies that are bound to the immobilized hormones on the plate are measured by adding Streptavidin-HRP, which binds to the biotin-labeled antibodies. To detect the HRP, TMB substrate is added, which produces a blue color when it interacts with HRP. After the blue color fully develops, the reaction is terminated with an acidic Stop solution, which changes the blue color to yellow. The intensity of the yellow color is measured at an absorbance of 450 nm and is used to determine the hormone level in the sample. Because this is a competitive ELISA assay, the signal is inversely proportional to the hormone concentration, since higher free hormone concentrations will prevent the labeled antibody from binding to the plate.

Materials Required but Not Provided

- Microplate reader capable of measuring absorbance at 450nm.

Materials Provided

- Insulin ELISA Plate (4°C)
- Dilution Buffer (4°C)
- 5x Assay Wash Buffer (4°C)
- Detection Antibody (-20°C)
- Streptavidin-HRP (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Culture Medium Preparation

1. Cell culture medium can be assayed directly or stored away at -80°C.

Cell Lysate Preparation

2. Wash the cells once with PBS before lysing the cells.
3. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
4. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
5. If necessary, homogenize the cell lysates with a sonicator.
6. The cell lysates may be assayed directly or stored at -80°C.
7. Use PBS to dilute the cell sample to the appropriate concentration for each assay, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration for each assay, if necessary.

Assay Procedure

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O.
- Dilute Detection antibody 50 times with 1x Diluent buffer.
- Dilute streptavidin-HRP 200 times with 1x Diluent buffer.

1. Add 100 µL of sample to each well and incubate for 1 hour at room temperature with gentle shaking.
2. 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. Make sure all liquid is removed after each wash.
3. Add 100 µL of diluted Detection antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
4. Repeat the aspiration/wash step in step 2.
5. Add 100 µL of diluted streptavidin-HRP to each well and incubate for 45 minutes at room temperature with gentle shaking.
6. Repeat the aspiration/wash step in step 2.
7. Add 100 µL of Substrate to each well and incubate for 10-30 minutes. **Substrate incubation time may vary due to differences in antibody reactivity. Stronger blue signals can be stopped earlier. Weaker signals can be incubated for a longer time up to 1 hour. Always stop the reactions of all samples from the same row at the same time.**
8. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Immediately measure the absorbance of the plate at 450 nm using a plate reader.