Signosis
Innovative Plate Assay Solutions

Human Anti-Insulin ELISA Kit

Catalog Number EA-5014 **(For Research Use Only)**

Introduction

Insulin is a hormone produced by beta cells in the pancreas. Beta cells secrete insulin in response to high glucose levels in the blood, and insulin helps to bring blood sugar levels back down by promoting glucose absorption and metabolism in cells. In diabetes mellitus, insulin activity is decreased or absent, which results in high blood sugar levels. There are two types of diabetes. In type 1 diabetes, beta cells are destroyed by the immune system through an autoimmune reaction. As a result, insulin cannot be produced by the pancreas, and blood sugar levels become elevated without treatment. In type 2 diabetes, beta cell activity is disrupted, but not completely eliminated like in type 1 diabetes. Type 2 diabetes is characterized by insulin resistance, in which cells are unresponsive to the concentration of blood glucose despite the secretion of insulin into the blood. Type 1 diabetes can distinguished from type 2 diabetes by testing for the presence of autoantibodies that target insulin.

Principle of the assay

The Human Anti-Insulin ELISA Kit detects anti-insulin antibodies in human serum. This kit utilizes a plate coated with insulin to immobilize the autoantibodies of interest. Anti-human IgG antibody conjugated to horseradish peroxidase (HRP) is used to label the insulin-bound antibodies, and the antibodies are detected by adding the HRP substrate, TMB, which forms a blue color in the presence of HRP. The color reaction is then terminated with Stop Solution, which causes the blue color to change to yellow. The autoimmune antibody concentration in each well is directly proportional to its color intensity and can be quantified by measuring its optical density at 450 nm (OD450) in a microplate reader.

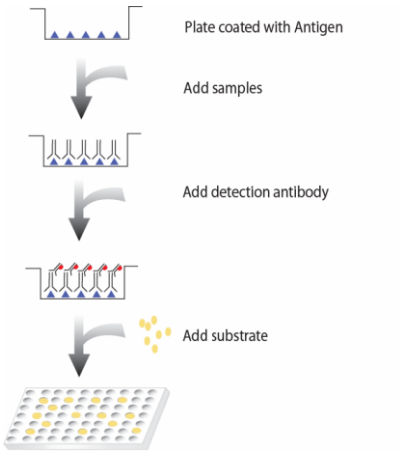

Diagram of Autoimmune ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-well strip Plate coated with histone	1	4°C
Anti-Human IgG antibody conjugated to HRP	10µL	4°C
1X Diluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Shaker

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 1000 times of anti-human IgG antibody conjugated to HRP with 1X Diluent buffer.

Storage and Preparation

Store all reagents at 2-8°C.
All reagents must be brought to room temperature (20-25°C) prior to use.
When stored at 2-8°C, the diluted Assay wash buffer is stable until the kit expiration date.

Precautions

Human blood derivatives and patient specimens should be considered potentially infectious. All human derived components need to be tested for the negative HBsAg, HCV, HIV-1 and 2 and HTLV-I. Follow good laboratory practices in storing, dispensing and disposing of these materials.

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2.Add 100 µl of diluted samples (1:100 diluted with 1X Diluent Buffer) per well and incubate for 1 hour at room temperature with gentle shaking. *Note: We recommend having a blank condition. For the blank, add only 1x Diluent buffer to the well.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process twice for a total of three washes. Completely remove liquid at each wash by firmly tapping the plate against clean paper towels.
4. Add 100µl of diluted anti-Human IgG antibody conjugated to HRP to each well and incubate for 30 minutes at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100µl of Substrate to each well and incubate for 7-30 minutes. *Note: Samples should be stopped when blue color begins to appear in blank.
8. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.