Signosis
Innovative Plate Assay Solutions

Mouse Anti-GAD65 ELISA Kit

Catalog Number EA-5211 **(For Research Use Only)**

Introduction

GAD65 is one of the two isoforms of glutamate decarboxylase (GAD), an enzyme that catalyzes the decarboxylation of glutamate to produce gamma-aminobutyric acid (GABA). This enzyme is primarily expressed in the brain but can also be found in the insulin-producing beta cells of the pancreas. Although the function of GAD65 in the pancreas is not well understood, it is believed to help modulate the secretion of glucagon in alpha cells. GAD65 is a target of autoantibodies in people who later develop type 1 diabetes or latent autoimmune diabetes. Because of this, GAD autoantibodies are often used as a biomarker for these conditions.

Principle of the assay

The Mouse Anti-GAD65 ELISA Kit detects anti-GAD65 antibodies in mouse serum. This kit utilizes a plate coated with GAD65 to immobilize the autoantibodies of interest. Anti-mouse IgG antibody conjugated to horseradish peroxidase (HRP) is used to label the GAD65-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.

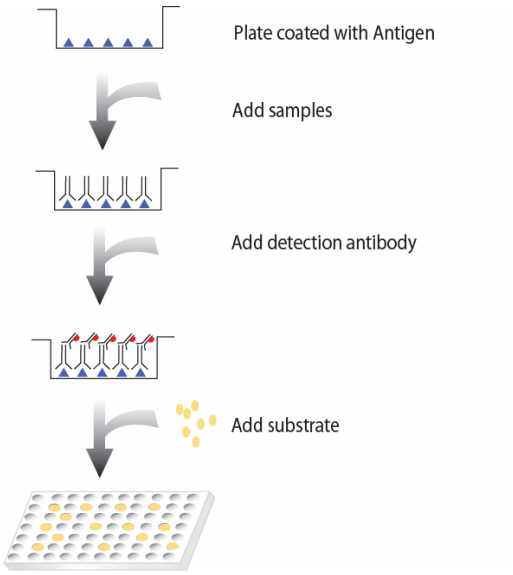

Diagram of Autoimmune ELISA

Materials provided with the kit

- 8x12 96-well plate coated with GAD65 (4ºC).
- Anti-mouse IgG antibody conjugated to HRP (4ºC).
- Mouse anti-GAD65 positive control (4ºC).
- 1X Diluent buffer (4ºC).
- 5X Assay wash buffer (4ºC).
- Substrate (4ºC).
- Stop Solution (4ºC)

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-mouse 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.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2. Standard Curve:
   - Add 200µl 1xDiluent Buffer to the 1st well on one strip
   - Add 100µl 1x Diluent Buffer to the rest of wells on the same strip
   - Add appropriate amount of mouse GAD65 positive control (50 µg/ml) to 1st well as 1st dilution
   - Mix 1st dilution in 1st well and transfer 100µl from 1st to next well for next dilution. Perform six two-fold serial dilutions
   - 1xDiluent buffer serves as the zero standard or blank

Note: The first dilution starting from 250ng/ml is recommended.

3.Add 100 µl of diluted samples or positive control (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.

4. 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.
5. Add 100µl of diluted anti-mouse IgG antibody conjugated to HRP to each well and incubate for 30 minutes at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 3.
7. Add 100µl of Substrate to each well and incubate for 5-15 minutes.
*Note: Positive control will turn blue. 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.