Creative Diagnostics®

Version 05-10/25

User's Manual

Mouse GAD-Ab-IgM (Glutamic Acid Decarboxylase Autoantibody IgM) ELISA Kit

REF **DEIA-JY25152**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Mouse GAD-Ab-IgM (Glutamic Acid Decarboxylase Autoantibody IgM) ELISA Kit is designed for the Semi-quantitative analysis of Mouse Serum, plasma samples.

General Description

GAD-Ab, or Glutamic Acid Decarboxylase Autoantibodies, are antibodies that target the glutamic acid decarboxylase (GAD) enzyme, which is crucial for producing GABA, an inhibitory neurotransmitter. These antibodies are associated with several autoimmune conditions, most notably Stiff Person Syndrome (SPS) and Type 1 Diabetes (T1D). Testing for GAD-Ab can help in the prediction, diagnosis, and management of these conditions, although their correlation with disease severity or therapeutic response is not always clear. Two vertebrate genes, GAD1 and GAD2, encode distinct GAD proteins: GAD67 and GAD65. GAD1 and GAD2 sequences are highly similar to each other, and they share a common intron-exon organization, indicating a common origin.

Principles of Testing

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the indirect principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for antibody. After the substrate reaction the intensity of the developed color is proportional to the amount of detected specific antibodies. Results of samples can be determined directly using the standard curve.

Reagents And Materials Provided

1. Polystyrene microwell ELISA plates coated with a purified antigen(12-1 x 8 wells), with holder in foil package containing desiccants
2. Negative Control, 1 vial of buffer 1.2 mL
3. Positive Control, 1 vial of buffer 1.2 mL
4. Calibrator A, 1 vial of buffer containing preservative, prediluted, 1.2 mL
5. Calibrator B, 1 vial of buffer containing preservative, prediluted, 1.2 mL
6. Calibrator C, 1 vial of buffer containing preservative, prediluted, 1.2 mL
7. Calibrator D, 1 vial of buffer containing preservative, prediluted, 1.2 mL
8. Sample Diluent, 1 vial – colored straw containing PBS-buffered saline, protein stabilizers and preservative, 50 mL.
9. Antibody Enzyme Conjugate, colored blue containing buffer, protein stabilizers and preservative, 12 mL
10. Wash Buffer (20 ×), 50 mL
11. TMB Chromogen, containing stabilizers, 10 mL
12. Stop Solution, Colorless, 10 mL

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37°C incubator (CO_2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10 μL, 5-50 μL, 20-200 μL, 200-1000 μL) and multi-channel pipette with disposable tips (calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Storage

2–8 °C, protected from light and moisture.

Specimen Collection And Preparation

This procedure should be performed with a serum or plasma specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Serum: Following collection, the serum should be separated from the clot.

1. Store samples at room temperature no longer than 8 hours.
2. If the assay will not be completed within 8 hours, refrigerate the sample at 2- 8°C.
3. If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Plasma: Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Reagent Preparation

Dilute 20× Wash Buffer 1:20 with distilled water (1 part buffer + 19 parts water).

Assay Procedure

Bring all reagents and samples to room temperature (20-25°C) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and controls be assayed in duplicate.

1. Prepare all reagents, and samples as directed in the previous sections.
2. To determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.

3. Set a Blank well without any solution.
4. Add 100 µl of Control, Standards or diluted Sample per well. Samples and controls must beassayed in duplicate. Cover with the adhesive strip provided. Incubate for 30 minutes at room temperature (20-25°C).
5. Aspirate each well and wash for a total of five washes. Wash by filling each well with Wash Buffer (300 µl) sing a squirt bottle, multi-channel pipette, manifold dispenser, or Auto washer, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µl of HRP-conjugate (1x) to each well (not to Blank!). Cover the microtiter plate with the adhesive strip. Incubate for 30 minutes at room temperature (20-25°C).
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 100 µl of TMB Substrate to each well. Incubate for 20 minutes at room temperature (20-25°C). Protect from light.
9. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
10. Take blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

*Samples may require dilution. Please refer to Specimen Collection And Preparation.

Quality Control

Refer to the COA for more information

• The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.

• The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Interpretation Of Results

Visually

Observe the colour intensity of the control and specimen wells 15 minutes after the addition of substrate TMB. The positive results should be blue which turns yellow after the addition of stopping solution. For quantification assays, a spectrophotometer is required for accurate results.

Photometrically

Read the microwell plate at 450 nm / 620 nm (or 450 nm if dual-wavelength is not available) in a compatible ELISA plate reader, blanked against air.

A calibration curve can be established by plotting standard concentration on the x-axis (linear scale) against the absorbance of the standards on the y-axis (linear scale). The vitellogenin concentrations can then be read off the calibration curve. A 4-parameter curve fit should be used for automatic data reduction. If samples were prediluted, the concentration will be obtained by multiplying the value read off the calibration curve by the dilution factor.

Detection Range

0 - 200 U

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.