Technical Manual

Human Glutamic Acid Decarboxylase (GAD) IgG ELISA Kit

- Catalogue Code: HDES0076
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the Glutamic Acid Decarboxylase (GAD) antibody (IgG) in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified GAD antigen. Samples are added to the ELISA Microtiter plate wells and the GAD-Ab in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugate is added to each well and react with the compound to form “GAD antigen- GAD antibody-HRP conjugate” compound. The TMB substrate is added to initiate the color developing reaction. The presence of GAD- IgG can be determined according to the OD value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Microtiter plate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper

4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the Microtiter plate Reader.
7. **Each reagent is optimized for use in the HDES0076. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0076 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

5. Storage and expiry date

Store unopened at 2-8° C. Do not freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the box.

6. Sample preparation

1. **Serum**: Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive. Samples can be stored at 2-8° C for one week and stored at -20° C for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
2. Anticoagulant (such as EDTA, sodium citrate and heparin, etc.) in samples do not affect the results. Samples with Sodium azide, hyperlipidemia, severe hemolysis, high concentration of proteins may lead to wrong results, and they are not recommended to be used.
3. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 1 well for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
   a) Add 100 µL of **Negative/Positive Control** respectively to 3 negative control wells, 1 positive control well, keep the blank control well empty.
   b) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (add 100 µL of Sample Diluent and add 10 µL of serum), mix fully.
3. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37° C in shading light..
4. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60s each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 µL of **HRP Conjugate** to each well except the blank control well, mix fully.
6. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37° C in shading light.
7. **Wash:** repeat step 4.
8. **Add Substrate:** add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 10 minutes at 37° C in shading light.
9. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
10. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection.

8. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: A450 ≤ 0.08;
positive control (PC): A_{450} > 0.50 and average A value of negative control (NC): A_{450} < 0.08.

9. Interpretation of test results

Cut Off(C.0) = 0.10 + average A value of negative control(NC) (when average A_{450} of NC < 0.05, calculate at 0.05; while average A_{450} of NC ≥ 0.05, calculate at the actual value).

1. Positive result: average A value of sample ≥ Cut Off.
2. Negative result: average A value of sample < Cut Off.

10. Limitations of test method

1. This test is only used as the qualitative detection of GAD- IgG antibodies in serum of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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