Technical Manual

Human Anti-β2-glycoprotein I (β2-GP Ⅰ) IgG Antibodies ELISA Kit

- Catalogue Code: HDES0031
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the Anti-β2-glycoprotein I (β2-GP Ⅰ) Antibodies in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified β2-GP Ⅰ antigen. Samples are added to the ELISA Microtiter plate wells and the β2-GP Ⅰ-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 "β2-GP Ⅰ antigen- β2-GP Ⅰ antibody-HRP conjugate" compound. The TMB substrate is added to initiate the color developing reaction. The presence of β2-GP Ⅰ-Ab can be determined according to the OD value after colorimetric assay with the Micro-plate reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	0.5 mL
Negative Control	0.5 mL
HRP Conjugated Working Solution	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
Manual	1 copy

3. Experimental instrument

- Micro-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
- Loading slot for Wash Buffer

4. Sample preparation

1. Serum can be used as detected sample. Fresh collected samples should be fully centrifuged, then take clear liquid for test. Anticoagulant (such as EDTA, sodium citrate and heparin, etc.) in samples do not affect the results.
2. Samples with sodium azide cannot be detected. Because the sodium azide may inhibit the HRP activity. The suspended fibrous protein may cause a false positive if not fully precipitated. Avoid of samples with hyperlipidemia, hemolysis or jaundice. Obviously contaminated samples can't be detected.
3. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them below -20° C and avoid freeze-thaw cycles.

5. Assay procedure

Bring all reagents to room temperature for 30 min. Dilute the 20×Concentrated Wash Buffer for 20 times with distilled water.

1. **Add sample:**
   (1) Take out Micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 1 well for positive control (100 µL control serum for each well). (Blank well is not necessary for dual-wavelength detection)
   (2) Dilute the tested serum with **Sample Diluent** at 1:10 (add 100 µL of Sample Diluent to the reaction well, and then add 10 µL of serum sample), mix fully.
   (3) Gently tap the plate to ensure thorough mixing.
2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
3. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
4. **HRP conjugate:** Add 100 µL of HRP Conjugate working solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
6. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
7. **Add substrate:** Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 10 min at 37° C in dark.
8. **Stop reaction:** Add 50 µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. **OD Measurement:** Set the Micro-plate 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 needed when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

6. Reference value

1. **Result analysis**
   (1) Use each test result independently. Determine the result according to the Cut Off value.
   (2) Calculate the Cut Off: Cut Off(C.0) = 0.10 + negative control (NC) average A value (when NC average A_{450} < 0.05, calculate at 0.05; while NC average A_{450} ≥ 0.05, calculate at the actual value).
2. **Quality control**
   (1) Blank well (just chromogenic agent and Stop Solution) absorbance ≤ 0.08.
   (2) Positive control (PC) A_{450} > 0.50.
   (3) Negative control (NC) A_{450} < 0.08.
   The experimental result is valid if quality control is valid.
3. **Determination of results**
   (1) Positive result: Sample absorbance ≥ Cut Off.
   (2) Negative result: Sample absorbance < Cut Off.

7. Interpretation of results

1. Negative result indicates there is no β2-GP Ⅰ antibody detected in samples, while positive result means the opposite.
2. The positive result of β2-GP Ⅰ antibody is an important auxiliary index of diagnosis of APS and other autoimmune diseases.

8. Limitations of test method

3. This test is only used as the qualitative detection of β 2-GP Ⅰ -Ab in serum of human.
4. 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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