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User's Manual

Mouse anti-β2 glycoprotein 1 IgM antibody ELISA kit

REF **DEIA250503**

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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

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PRODUCT INFORMATION

Intended Use

This kit is intended for quantitative detection of mouse anti-β2GP1 IgM antibodies in serum, plasma, or cell culture supernatants. For research use only.

Principles of Testing

This kit employs an indirect two-step ELISA. β2GP1 protein (solid-phase antigen) is pre-coated on microplate wells. After adding standards/samples and incubating, unbound components are washed away. HRP-labeled anti-mouse IgM is added, forming a "solid-phase antigen–antibody–enzyme conjugate" complex. Upon adding substrates A and B, HRP catalyzes a colorimetric reaction (blue → yellow after stopping). The OD450 is proportional to β2GP1 IgM concentration.

Reagents And Materials Provided

1. Pre-coated Microplate: 96 wells
2. Antibody-based standards: 0.3 mL × 6 (0, 5, 10, 20, 40, 80 ng/mL)
3. HRP-labeled Antibody: 10 mL
4. Substrate A: 6 mL, Hydrogen peroxide solution
5. Substrate B: 6 mL, TMB solution
6. Sample Diluent: 60 mL
7. Stop Solution: 6 mL
8. 20× Wash Buffer: 30 mL, PBS with 0.15% Tween 20
9. Ziplock Bag: 1
10. Adhesive Seals: 2

Notes:

1. Verify component labels and quantities before use.
2. If reusing components, ensure no prior contamination.
3. Re-seal unused microplates and store at 2-8°C.

Materials Required But Not Supplied

1. ELISA plate reader (450 nm).
2. Automated plate washer.
3. Shaker.
4. Adjustable pipettes and tips (multi-channel recommended for high-throughput).

Storage

Store unopened kits at 2–8°C; valid for 6 months. Do not use expired kits.

Specimen Collection And Preparation

The following is a general guide for sample collection and storage. Sodium azide should not be used as a preservative during all sample collection and storage processes. If samples are not analyzed immediately, they should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

1. **Cell Culture Supernatant:** Centrifuge to remove precipitates, analyze immediately or aliquot and store frozen at -20°C.
2. **Serum:** Collect blood in a clean test tube, allow it to clot at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect the serum. Analyze immediately or aliquot and store frozen at -20°C.
3. **Plasma:** Use heparin, citrate, or EDTA for anticoagulation. Centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes after blood collection. To eliminate the influence of platelets, it is recommended to further centrifuge at 10000×g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.
4. **Cell Lysate:** For adherent cells, remove the culture medium and wash once with PBS, normal saline, or serum-free culture medium. Add an appropriate amount of lysis buffer and pipette up and down several times to ensure full contact between the lysis buffer and the cells. Cells will usually be lysed after 10 seconds. For suspension cells, collect the cells by centrifugation and wash once with PBS, normal saline, or serum-free culture medium. Add an appropriate amount of lysis buffer, resuspend the cells by pipetting, and gently flick with your fingers to fully lyse the cells. After complete lysis, centrifuge at 10000-14000×g for 3-5 minutes and collect the supernatant. Analyze immediately or aliquot and store frozen at -20°C.
5. **Urine:** Collect in a sterile tube and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If precipitation forms, centrifuge again.

Reagent Preparation

1. Before use, all components should be allowed to equilibrate to room temperature for at least 120 minutes to ensure they are fully warmed.
2. **20× Wash Buffer:** Crystals may form in the concentrated wash buffer when taken out of the refrigerator, which is a normal phenomenon. Heat in a water bath until the crystals are completely dissolved. Dilute the concentrated wash buffer with distilled water at a ratio of 1:20 (i.e., 1 part concentrated wash solution to 19 parts distilled water).
3. **Substrate:** Mix substrate A and B thoroughly in a 1:1 volume ratio before use. Use within 15 minutes after mixing.

Assay Procedure

It is recommended that users first conduct a preliminary experiment to determine the optimal sample dilution factor, and then conduct the formal experiment.

1. Allow all reagents and components to reach room temperature. It is recommended to run duplicates for standards and samples.
2. Take out the required strips from the aluminum foil bag. Seal the remaining strips in a ziplock bag and return them to the refrigerator.

3. Set up standard wells, sample wells, and blank wells. Add 50 µL standards and samples. Do not add anything to the blank wells. Cover the reaction plate with sealer and incubate at 37°C for 30 minutes.
4. Remove the plate sealer, discard the liquid, and pat dry on absorbent paper. Fill each well with wash buffer, let it stand for 20 seconds, discard the wash buffer, and pat dry on absorbent paper. Repeat this process 4 times (for a total of 5 washes). If using an automatic plate washer, please follow the plate washer's operating procedure and add a 20-second soak program, which can improve the detection accuracy. After washing, before adding the substrate, thoroughly pat dry the reaction plate on clean, lint-free paper.
5. Except for the blank wells, add 100 µL HRP-conjugated antibody to the standard and sample wells.
6. Cover the reaction plate with sealer and incubate at 37°C for 30 minutes, protected from light.
7. Repeat washing step (step 4).
8. Mix substrates A and B thoroughly in a 1:1 volume ratio. Add 100 µL substrate mixture to all wells. Cover the reaction plate with sealer and incubate at 37°C for 15 minutes, protected from light.
9. Add 50 µL stop solution to all wells. Read the absorbance (OD) of each well on an ELISA reader at 450nm.

Calculation

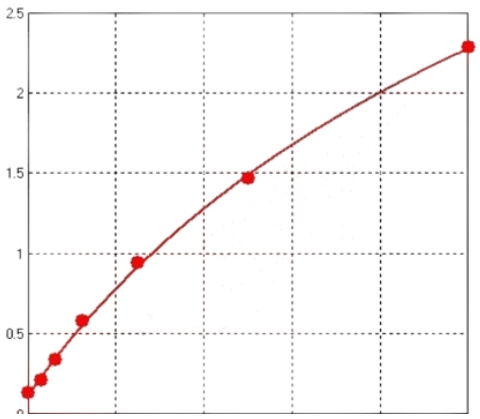
Using the standard concentrations as the x-axis and the corresponding absorbance (OD values) as the y-axis, use computer software to perform a four-parameter logistic (4-PL) curve fit to create a standard curve. Calculate sample concentrations using ELISA software.

Multiply by dilution factor if applicable.

Typical Standard Curve

For reference only

Standard Curve Linearity: Correlation coefficient (r) ≥ 0.9900.



Precision

Intra-assay CV% < 10%

Inter-assay CV% < 15%

Detection Range

2.5-80 ng/mL

Detection Limit

0.1ng/mL

Specificity

Recognizes natural and recombinant mouse anti-β2GP1 IgM antibodies without cross-reactivity with structural analogs.

Recovery

85%-115%

Precautions

1. All liquid components should be clear and transparent, without precipitation or flocculent matter. The microplate aluminum foil bag should be vacuum-packed, without damage or leakage.
2. Please wear lab coats and latex gloves for protection during the experiment. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.
3. Strictly follow the specified time and temperature for incubation to ensure accurate results. All reagents must reach room temperature (20-25°C) before use. Refrigerate reagents immediately after use.
4. Incorrect plate washing can lead to inaccurate results. Ensure that the liquid in the wells is aspirated as much as possible before adding the substrate. Do not let the microwells dry out during incubation.
5. Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
6. The substrate chromogenic solution should be colorless or very light in color.
7. Avoid cross-contamination of reagents and specimens to avoid erroneous results.
8. Avoid direct exposure to strong light during storage and incubation.
9. Allow the sealed bag to equilibrate to room temperature before opening.
10. No reaction reagent should come into contact with bleaching solvent or strong gas emitted by bleaching solvent. Any bleaching component will destroy the biological activity of the reaction reagents in the kit.
11. The ELISA reader used for detection needs to be equipped with a filter that can detect a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat it 15 minutes before use.
12. Do not mix or substitute reagents in this kit with reagents from other batches or other sources.
13. The EP tubes and tips used in the experiment are for single use only and are strictly prohibited from being mixed.
14. Do not use expired reagents.
15. The final experimental results are closely related to the effectiveness of the reagents, the operator's relevant operations, and the experimental environment at the time. CD is only responsible for the kit itself and is not responsible for sample consumption caused by the use of the kit. Users are requested to fully consider the

possible amount of sample usage before use and reserve sufficient samples.

Limitations

1. For research use only.
2. Do not mix components from other kits.
3. Use the provided sample diluent.
4. Dilute samples exceeding the upper detection limit.
5. Heterophilic antibodies (e.g., human anti-mouse) may interfere.
6. Results from other methods are not directly comparable.