Version 04-06/24

User's Manual

Human Ceramide Synthase 3 (CERS3) ELISA Kit

REF **DEIA-JY2305**

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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 immunoassay kit allows for the in vitro quantitative determination of target antigen concentrations in serum, plasma, tissue homogenates, cell culture supernates or other biological fluids.

Principles of Testing

The microtiter plate provided in this kit has been pre-coated with an antibody specific to target antigen. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for target antigen and then avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated.Then a TMB substrate solution is added to each well. Only those wells that contain target antigen, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color.The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 2nm.The concentration of target antigen in the samples is then determined by comparing the O.D.of the samples to the standard curve.

Reagents And Materials Provided

1. ELISA Microplate: 8×12, -20°C
2. Lyophilized Standard: 2 vial, -20°C
3. Sample Dilution Buffer: 20 ml, -20°C
4. Assay Diluent A: 1 vial, 10 ml, -20°C
5. Assay Diluent B: 1 vial, 10 ml, -20°C
6. Detection Reagent A: 120 µl, -20°C
7. Detection Reagent B: 120 µl, -20°C
8. Substrate: 10 ml, 2-8°C
9. Stop Solution: 10 ml, 2-8°C
10. Wash Buffer (25×): 30 ml, 2-8°C
11. Plate Sealer: 5 pieces
12. Product Description: 1 copy

Specimen Collection And Preparation

1. **Serum:** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000 ×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
2. **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.

3. **Tissue Homogenates:** As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01 M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an µLtrasonic cell disrupter or subject it to freeze -thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 ×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
4. **Adherent and Suspension Cell Culture:** Use three T25 flasks or one T75 flask for cell culture, the number of cells ($1x10^7$);

   Suspension cell: centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant;

   Adherent cell: collect supernatant directly; centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant for immediate detection or store it separately at -80°C.
5. **Cell Lysate Preparation:**

   Two types of cell lysates are specified below.

   Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes; Then add pre-cooling PBS into collected cell and gently mix. Recollect cell by repeating centrifugation. Add 0.5-1 ml RIPA lysis buffer (NP-40 lysis buffer or Triton X100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Lyse the cell on ice for 0.5-1 h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic cell disruptor (300W, 3-5s/time, 30s intervals, four-five times) or ultrasonic generator (14 µm for 30s). At the end of lysate or µLtrasonic disruption, centrifuge at 10000 rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.

   Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS once. Then, add 0.5-1 ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add the suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Scrape adherent cell gently with a cell scraper. Add the cell suspension into centrifugal tube. Lyse the cell on ice for 0.5-1 h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by µ Ltrasonic generator (14 µm for 30s) or µLtrasonic cell disruptor (300W, 3-5s/time, 30s intervals, four-five times). At the end of lysate/ultrasonic disruption, centrifuge at 10000 rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.
6. **Other Biological Fluids:** Centrifuge samples for 20 minutes at 1000 ×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Reagent Preparation

Standard-Please refer to the Data Sheet inserting in the kit. Detection Reagent A and B-Dilute to the working concentration using Assay Diluent A and B(1:100), respectively.

Wash Buffer-If crystals have formed in the concentrate,warm to room

temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Please accurately prepare the working solutions on demand before use.

Calculation

Average the duplicate readings for each standard, control,and sample,then subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.As an alternative,construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentrations versus the log of the O.D, and the best fit line can be determined by regression analysis. It is recommended to use some professional software to do this calculation,such as Curve Expert. This procedure will produce an adequate but less precise fit of the data.If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Detection Range

0.312 - 20 ng/mL

Sensitivity

0.155 ng/mL

Precautions

1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved.The reconstituted Standards, Detection Reagent A and B can be used only once.
2. To ensure accurate results,proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.
3. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions,and between reagent additions. Also, use separate reservoirs for each reagent.
4. The wash procedure is critical.Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Substrate Solution is easily contaminated.Please protect it from light.
6. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to printed instruction inside in the kit.