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Human Alkaline Sphingomyelinase (ALK-SMASE) ELISA Kit

Cat.No: MBS039905

Store All Reagents At 2-8°C !
Package Size: 96 T/Kit
Valid Period: Six Months (2-8°C)

For Specimens:
Serum, Plasma, Cell Culture Supernatants, Tissue Homogenate, Urine and other Body Fluids

FOR RESEARCH USE ONLY!
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

CONTENT:

1. INTRODUCTION

ELISA is a simple and highly sensitive method of analysis that allows for simultaneous and rapid quantification of a large number of specimens. The assay is based on the specific recognition of the target compound (analyte/antigen) by antibodies which bind to the compound. The antigen-antibody complex is detected and measured with the aid of an enzyme-labeled antibody or antigen. Upon addition of a non-colored reagent, the enzyme produces a color reaction where the color intensity is directly or inversely proportional to the concentration of the analyte in the specimen.

2. INTENDED USE

This ELISA kit is intended for laboratory in vitro research use only, not for drug, household or other use! The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm by a spectrophotometer. In order to measure the concentration of Human ALK-SMASE in the specimen, this ELISA kit includes a set of calibration standards. The calibration standards are assayed at the same time as the specimens and allow the operator to produce a standard curve of Optical Density versus Human ALK-SMASE concentration. The concentration of Human ALK-SMASE in the specimens is then determined by comparing the O.D. of the specimens to the standard curve.

3. SPECIMENS COLLECTION AND STORAGE

Serum - Allow specimens to clot for two hours at room temperature or overnight at 2 - 8°C before centrifugation for 20 minutes at approximately 1000 × g (or 3000 rpm). Assay immediately or store specimens in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge specimens for 15 minutes at 1000 × g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. Assay immediately or store specimens in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Cell lysates - Cells should be lysed according to the following directions. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly. Wash cells three times in PBS (0.02mol/L, pH 7.2-7.4). Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20 °C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times. Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8 °C to remove cellular debris. Assay immediately or store specimens at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

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Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02mol/L, pH 7.2-7.4). Remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS (Usually 10mg tissue to 100μl PBS.) with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, centrifugate homogenates for 15 minutes at 1500×g (or 5000 rpm). Assay immediately or store specimens at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Cell culture supernatants - Centrifuge cell culture supernatants at 1000 × g (or 3000 rpm) for 15 minutes to remove debris. Collect the supernatants carefully, assay immediately or store specimens at -20°C or -80°C.

Feces - Collect by sterile tube. Dilute the specimens in a certain amount of PBS (0.02mol/L, pH 7.2-7.4). Usually 10mg feces to 100μl PBS. Fully shaking and after 10 minutes' standing, Centrifuge specimens for approximately 20 minutes at 1000 × g (or 3000 rpm). Collect the supernatants carefully, assay immediately or store specimens at -20°C or -80°C.

Urine and other Body Fluids - Collect by sterile tube. 1000 × g (or 3000 rpm) for approximately 20 minutes. Collect the supernatants carefully, assay immediately or store specimens at -20°C or -80°C. When sediments occurred during storage, centrifugation should be performed again. When collecting **Pleuroperitoneal Fluid** and **Cerebrospinal Fluid**, please follow the procedures above-mentioned.

Note:

1. Specimens to be used within 5 days may be stored at 4°C, otherwise specimens must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.

2. Specimen hemolysis will influence the result, so the specimens should be centrifugated adequately and no hemolysis or granule was allowed.

3. When performing the assay, bring specimens to room temperature.

4. MATERIALS REQUIRED BUT NOT SUPPLIED

1) Standard microplate reader (450nm).

2) Precision pipettes and Disposable pipette tips.

3) Incubator (37°C).

4) Distilled or deionized water.

5. MATERIALS SUPPLIED

Items	Materials	48 Tests	96 Tests
1	Microelisa Stripplate	12×4 Strips	12×8 Strips
2	Standards (6 vial)	0.5ml×6 vials	0.5ml×6 vials
3	Specimen Diluent	3.0ml	6.0ml
4	HRP-Conjugate Reagent	5.0ml	10.0ml
5	20×Wash Solution	15ml	25ml
6	Stop Solution	3.0ml	6.0ml
7	Chromogen Solution A	3.0ml	6.0ml
8	Chromogen Solution B	3.0ml	6.0ml
9	Closure Plate Membrane	2	2
10	Sealed Bags	1	1
11	Instruction	1	1

Note: The concentration gradients of Standards are followed by: 200,100,50,25,12.5,6.25 U/L.

6. PRECAUTIONS

1) Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.

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2) Leave kit reagents and materials to reach room temperature (20-25°C.) naturally before use. Do not use water baths to thaw specimens or reagents.

3) Do not use kit components beyond their expiration date.

4) Use only deionized or distilled water to dilute reagents.

5) Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.

6) Use fresh disposable pipette tips for each transfer to avoid contamination.

7) Do not mix acid and sodium hypochlorite solutions.

8) Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

9) All specimens should be disposed of in a manner that will inactivate viruses.

10) Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

11) Substrate Solution is easily contaminated, they should be colourless or light-colored. So if bluish, please do not use them.

12) Keep Chromogen Solution B away from sources of heat or flame.

7. REAGENT PREPARATION AND STORAGE

Wash Solution (1×) - Dilute one volume of Wash Solution (20×) with nineteen volumes of deionized or distilled water. Wash Solution is stable for one month at 2-8°C.

8. ASSAY PROCEDURE

1) Prepare all reagents before starting assay procedure. It is recommended that all Standards and specimens be added in duplicate to the Microelisa Stripplate.

2) Set standard wells and testing specimen wells, add standard 50µl to each standard well. add specimen 50µl to each testing specimen well. **Blank well add nothing**.

3) Add 100µl of HRP-conjugate reagent to each well **except the blank well**, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4) Wash the Microtiter Plate 4 times.

Manual Washing - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1×), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. **Note**: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1×). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µl/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5) Add Chromogen Solution A 50µl and Chromogen Solution B 50µl to **each well** successively. Gently mix and then **protect from light** to incubate for 15 minutes at 37°C.

6) Add 50µl Stop Solution to **each well**. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7) Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

9. CALCULATION OF RESULTS

1) This standard curve is used to determine the amount in an unknown specimen. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.

2) First, calculate the mean O.D. value for each standard and specimen. All O.D. values are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.

3) To determine the amount in each specimen, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.

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4) Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain him own standard curve.

5) If specimens have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

6) If specimens generate values higher than the highest standard, dilute the specimens and repeat the assay.

10. THE PERFORMANCE OF THIS KIT

Detection Range: The detection range of this kit is 6.25 U/L ~ 200 U/L.

Sensitivity: The sensitivity of this kit is less than 1.0 U/L.

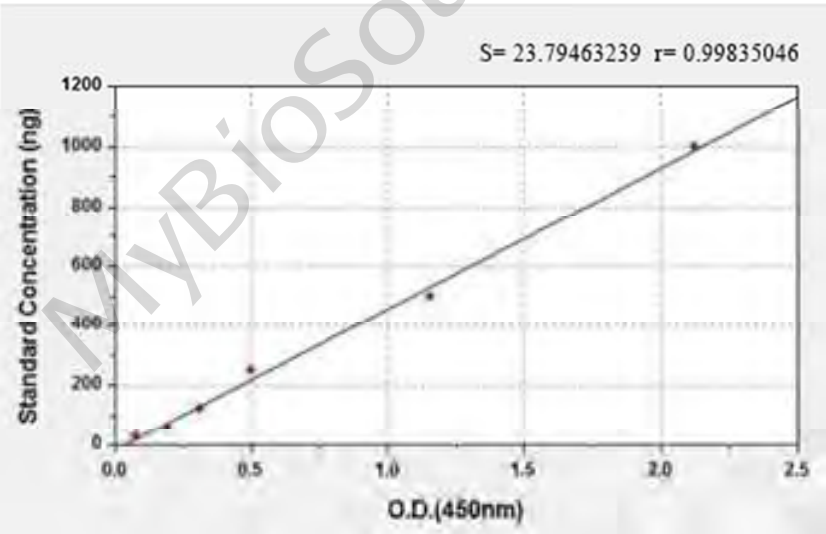
Specificity: This kit recognizes recombinant and natural Human ALK-SMASE, no significant cross-reactivity or interference was observed.

Reproducibility: Intra-assay CV (%) and Inter-assay CV (%) are less than 15%.

11. TYPICAL STANDARD CURVE

Reference in general, not for this kit in particular.

Standard Concentration	Mean O.D.(450nm)	Adjusted
0	0.043	None
31.2	0.121	0.078
62.5	0.236	0.193
125	0.355	0.312
250	0.541	0.498
500	1.198	1.155
1000	2.169	2.126



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