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Sunlong Medical™ Mouse Sphingosine Kinase 1 (SPHK1) ELISA Kit

Catalog Number: EL1815Mo

Size: 48 Test / 96 Test

Storage: 2 - 8℃

Validity Period: 6 months

Sensitivity: 0.062 ng/mL

Assay range: 0.3125 - 10 ng/mL

For quantitative in vitro detection of Mouse SPHK1 in cultured cells, serum, plasma and other samples.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Assay Principle

This kit employs double antibody sandwich ELISA technology: **Capture Antibody** is coated on the microplate to capture SPHK1 from samples and standards. After washing, biotin-labeled **Detection Antibody** is added and incubated, followed by washing to form a "**Capture Antibody-Antigen-Detection Antibody**" immune-complex. Subsequently, Streptavidin-Horseradish Peroxidase (**SA-HRP**) is added and incubated. After incubation and washing, **TMB Substrate** is added for color development. If the target analyte is present in the sample, a blue color develops. **Stop Solution** is then added to terminate the reaction. During the assay, unbound components are washed away. The **Optical Density** (OD) is measured at 450 nm using a microplate reader. The intensity of the color is proportional to the SPHK1 concentration in the sample, and the concentration is calculated by plotting a standard curve.

Precautions

1. **This kit is for research use only. Not for use in diagnostic procedures.**
2. After opening the sealed bag containing the pre-coated strips, any unused strips should be immediately resealed in a ziplock bag with desiccant. To minimize inter-assay variation, strips can be stored at 2-8°C if the next assay is within 48 hours. For longer intervals, store at -20°C and re-run the standard curve in the next assay.
3. The substrate chromogen solution TMB provided in the kit should remain colorless or very light in color before use until added to the plate. If the received chromogen solution exhibits an intense blue color, please contact technical support.
4. **The Stop Solution provided in this kit is a dilute sulfuric acid solution, which is corrosive. Handle with care. If contact with skin occurs, wipe off immediately and rinse thoroughly with water.**
5. **Operate strictly according to the instructions. If in doubt, please confirm with technical support before proceeding to avoid waste of samples and time.**
6. Do not mix reagents from different lot numbers. Do not use reagents from other sources or manufacturers.
7. Seal plates, absorbent paper, EP tubes, and pipette tips used during sample addition are for single use only. Do not reuse.

Kit Components

Component	96T Config	48T Config	Storage Condition
Pre-coated Plate	96 wells	48 wells	2-8°C
Standard (lyophilized)	2 vials	2 vials	
Reconstitution Solution	2 vials	2 vials	
Standard & Sample Diluent	25mL ×1 bottle	25mL ×1 bottle	

Biotinylated Ab	10mL ×1 bottle	10mL ×1 bottle	
SA-HRP	10mL ×1 bottle	10mL ×1 bottle	
TMB Substrate	10mL ×1 bottle	10mL ×1 bottle	
Stop Solution	6mL ×1 bottle	6mL ×1 bottle	
20× Wash Buffer	25mL ×1 bottle	25mL ×1 bottle	
Plate Sealer	4 sheets	4 sheets	RT
Instruction	1 copy	1 copy	

Materials Required but Not Provided:

Equipment & Instruments	Other Materials
Microplate reader with 450 nm filter	Absorbent paper or lab wipes
Pipettes of various volumes	Distilled or deionized water
Incubator capable of maintaining 37°C	Various pipette tips and EP tubes

Sample Collection and Handling:

1. **Serum:** Collect whole blood into serum separation tubes. Allow clotting at room temperature for 30 minutes to 2 hours. Centrifuge at 2-8°C, 2500-3500 × g for 20 minutes. Carefully collect the supernatant.
2. **Plasma:** Use EDTA or citrate as anticoagulant as required. Centrifuge samples within 30 minutes of collection at 2-8°C, 3000 × g for 15 minutes. Carefully collect the supernatant. If precipitation occurs during storage, re-centrifuge.
3. **Urine:** Collect using sterile tubes. Centrifuge at 2-8°C, 2500-3500 × g for 10 minutes. Carefully collect the supernatant. If precipitation occurs during storage, re-centrifuge. Handle pleural fluid, ascitic fluid, and cerebrospinal fluid similarly.
4. **Cell Culture Supernate:** Collect the fluid and centrifuge at 2-8°C, 2500-3500 × g for 20 minutes to remove impurities and cell debris. Collect the supernatant for assay.
5. **Cell Lysate:** Wash adherent cells gently with ice-cold PBS (0.01M, pH=7.4), then digest with trypsin. Collect cells by centrifugation at 2-8°C, 1000 × g for 5 minutes. Suspension cells can be collected directly by centrifugation. Wash collected cells 3 times with cold PBS. Resuspend 1×10^6 cells in 150-200μL PBS. Lyse cells by repeated freeze-thaw cycles or ultrasonication (it is recommended to add protease inhibitors to PBS; if the target concentration is expected to be low, reduce the PBS volume). Centrifuge the lysate at 2-8°C, 10000 × g for 10 minutes. Collect the supernatant for assay.
6. **Tissue Samples:** Rinse the tissue with ice-cold PBS (0.01M, pH=7.4) to remove residual blood. Weigh and mince the tissue. Add the minced tissue to an appropriate volume of

PBS (generally a 1:9 weight/volume ratio, e.g., 1g tissue to 9mL PBS; the volume can be adjusted according to experimental needs and should be recorded. It is recommended to add protease inhibitors to PBS) in a homogenizer. Grind thoroughly on ice. To further lyse cells, subject the homogenate to repeated freeze-thaw cycles or ultrasonication. Finally, centrifuge the homogenate at 2-8°C, 10000 × g for 5-10 minutes. Collect the supernatant for assay.

7. **Other Biological Samples:** Centrifuge at 2-8°C, 2500-3500 × g for 20 minutes. Carefully collect the supernatant.

Sample Appearance: Samples should be clear and transparent. Suspended matter should be removed by centrifugation.

Sample Storage: Assay samples as soon as possible after collection. If testing cannot be performed immediately, aliquot the samples and store at -20°C (test within 1 month) or -80°C (test within 6 months). Avoid repeated freeze-thaw cycles.

Reagent Preparation

Before use, equilibrate all reagents at room temperature or in a 37°C incubator for approximately 30 minutes.

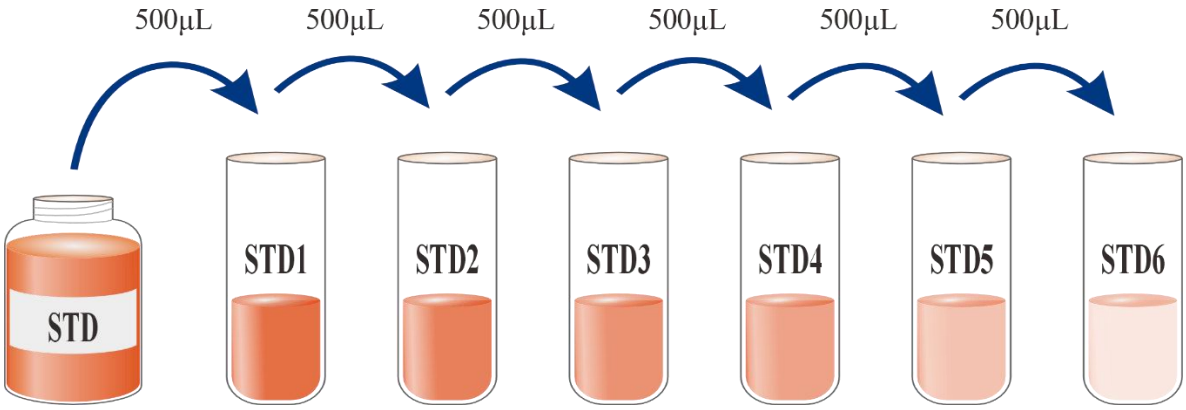
Wash Buffer Preparation: If crystals have formed in the Wash Buffer (20×), heat it at 37°C until the crystals are completely dissolved. Dilute 1:20 with distilled water (e.g., add 1 mL of concentrated wash buffer to 19 mL of distilled water).

Standard Reconstitution: Reconstitute the standard by adding the entire contents of one vial of Standard Reconstitution Solution to one vial of lyophilized standard powder. Gently invert the vial several times to mix (avoid vigorous shaking to prevent excessive bubbling). Allow the solution to stand for approximately 10 minutes. The concentration of the reconstituted standard stock solution (STD) is 20ng/mL.

Standard Serial Dilution: Prepare 6 test tubes and add 500 µL of Standard Diluent into each tube. Perform a two-fold serial dilution of the reconstituted standard stock solution (STD) across these 6 separate tubes to create 6 gradients, resulting in a total of 6 standard concentrations. The concentrations are as follows: 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, and 0.3125ng/mL.

The specific dilution method is as follows:

Transfer 500 µL from the higher concentration standard solution into the next tube, mix gently by pipetting, and repeat this process sequentially for the serial dilution (as shown in the diagram). Use Diluent as the zero standard(0ng/mL).



Concentrations after dilution are as follows (Unit: ng/mL)

STD	STD1	STD2	STD3	STD4	STD5	STD6
20	10	5	2.5	1.25	0.625	0.313

Note: If the SPHK1 concentration in the sample is higher than the highest standard point, dilute the sample with an appropriate dilution factor as needed and re-assay. Multiply the result by the dilution factor.

Assay Procedure

All standards and samples are recommended to be assayed in duplicate.

1. **Microplate Preparation:** Determine the number of strips required for the assay. Remove other unused strips and immediately return them to the sealed bag.
2. **Sample Incubation:** First, add 50 μL of sample dilution buffer to all wells. Then, add 50 μL of standards at varying concentrations to the standard wells, add another 50 μL of sample dilution buffer to the blank wells, and add 50 μL of the test samples to the sample wells. Cover the plate with a sealing film and incubate at 37°C for 1 hour. After incubation, add 200 μL of 1× wash buffer to each well, gently shake for 30 seconds, and discard the solution. Repeat this washing process three times, then blot dry.
3. **Antibody Incubation:** Add 100 μL of Biotinylated Antibody to each well. Cover the plate with a sealer and incubate at 37°C for 1 hour. After incubation, repeat the washing procedure as described in Step 2 three times and blot dry.
4. **Enzyme Conjugate Incubation:** Add 100 μL of SA-HRP to each well. Cover the plate with a sealer and incubate at 37°C for 30 minutes, protected from light. After incubation, repeat the washing procedure as described in Step 2 four times, and **blot dry on fresh absorbent paper.**
5. **Substrate Development:** Add 100 μL of TMB Substrate Solution to each well. Cover the plate with a sealer and incubate at 37°C for 15 minutes, protected from light.

6. **Stop Reaction:** After the color development, add 50µL of Stop Solution to each well. Gently tap the plate to mix. Measure the Optical Density (OD) at 450 nm using a pre-warmed microplate reader within 10 minutes.
   (Monitor the Substrate Development closely. If the STD1 well develops intense color, the reaction may be terminated early once a faint blue color appears in the STD6 well.)

Calculation of Results

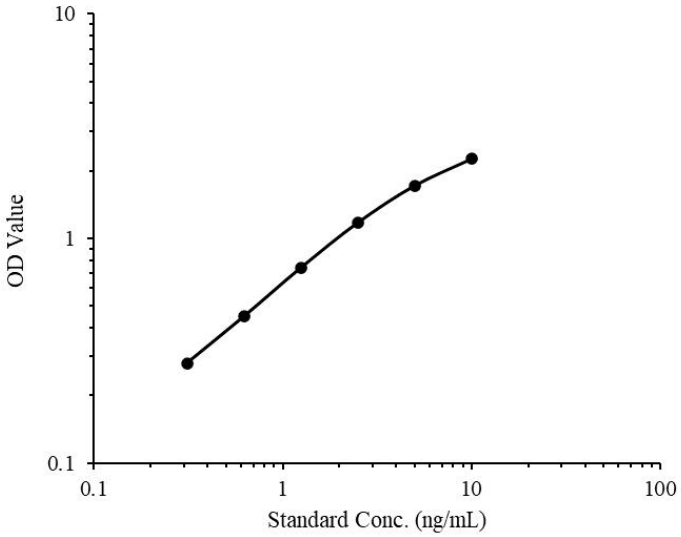
Calculate the average OD value for each standard and sample duplicate, then subtract the average OD value of the zero standard (blank) well to obtain the corrected OD value. Plot the corrected OD values (y-axis) against the corresponding standard concentrations (x-axis) on graph paper using a four-parameter logistic (4-PL) curve fit (omit the zero standard value when plotting). Alternatively, use computer software capable of generating a four-parameter logistic (4-PL) curve fit to create the standard curve.

If the OD value of a sample is higher than the highest standard point, the sample should be appropriately diluted and re-assayed. Multiply the concentration obtained from the standard curve by the dilution factor.

Typical Data

The following data and curve are for reference only. The experimenter must establish a standard curve based on their own experimental data.

Standard Conc. (ng/mL)	10	5	2.5	1.25	0.625	0.313	0
OD Value	2.708	1.532	0.794	0.425	0.222	0.132	0.038
Corrected OD Value	2.67	1.494	0.756	0.387	0.184	0.094	0



The standard curve shown in this figure is for example purposes only. Use the standard curve generated from the standards in the same assay to calculate sample results.

Precision

Intra-assay and inter-assay Coefficients of Variation (CV) are both <10%.

- **Intra-assay Precision:** Three known concentration samples were assayed 20 times on one plate. The CV of the concentrations was calculated.
- **Inter-assay Precision:** Three known concentration samples were assayed in 20 replicates across three different plates. The CV of the concentrations was calculated.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20

Mean (ng/mL)	0.62	1.25	2.5	0.62	1.25	2.5
Standard Deviation	0.011	0.025	0.083	0.015	0.036	0.076
CV (%)	1.71	2.03	3.33	2.49	2.87	3.05

Recovery

Recovery was tested by spiking known concentrations of Mouse SPHK1 into different sample matrices. The recovery range and average recovery are shown below.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	90-99	95
Plasma (n=8)	92-103	97
Cultured Cells (n=8)	100-118	105

Sensitivity

The minimum detectable concentration (sensitivity) of Mouse SPHK1, determined by testing samples, is 0.062ng/mL.

Linearity

High concentration Mouse SPHK1 was spiked into samples and then serially diluted 2-fold, 4-fold, 8-fold, and 16-fold within the range of the standard curve to assess linearity. Recovery rates and average recovery are shown below.

Dilution		Serum (n=4)	Cultured Cells (n=4)
1:2	Recovery Range (%)	95-110	94-107
	Avg. Recovery (%)	99	100
1:4	Recovery Range (%)	100-114	98-114
	Avg. Recovery (%)	102	106
1:8	Recovery Range (%)	101-117	102-114
	Avg. Recovery (%)	106	111

1:16	Recovery Range (%)	106-120	105-121
	Avg. Recovery (%)	110	118

Specificity

This kit specifically detects recombinant Mouse SPHK1. Other related proteins were prepared at 50 ng/mL in the dilution buffer and tested for cross-reactivity. No significant cross-reactivity was observed.