（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F079

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.35-40 μmol/L

Elabscience® Sphingomyelin (SM) Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

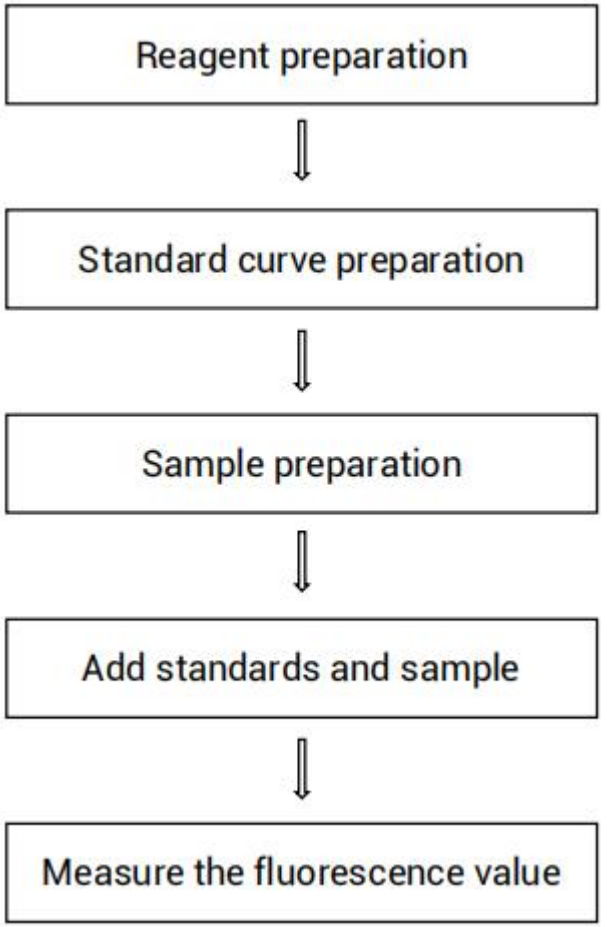
Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure sphingomyelin (SM) content in serum, plasma, animal tissue and cell samples.

Detection principle

Sphingomyelin (SM) is widely present in cell membranes and plasma lipoproteins. Metabolism of SM is regulated by sphingomyelin synthase and sphingomyelase, whose metabolites include ceramide, sphingosinol and choline phosphate, which are important second messengers. Studies have shown that SM and its metabolites play an important role in liver, lung, and cardiovascular diseases. The principle of this kit is that SM is catalyzed by sphingomyelin enzyme, and its products generate fluorescent substances with the probe under the action of other enzymes. By substituting the fluorescence value into the standard curve, SM content in the sample can be calculated.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Extraction Solution	25 mL × 1 vial	-20°C, 12 months
Reagent 3	Enzyme Reagent A	0.02 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Enzyme Reagent B	0.05 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Enzyme Reagent C	Powder × 2 vials	-20°C, 12 months shading light
Reagent 6	Probe	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 7	1 mmol/L Standard	0.5 mL × 1 vial	-20°C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Keep enzyme reagent A, enzyme reagent B and enzyme reagent C on ice during use. Equilibrate other reagents to 25℃ before use.

② The preparation of enzyme A working solution：
Before testing, please prepare sufficient enzyme A working solution. For example, prepare 2012 μL of enzyme A working solution (mix well 2000 μL of buffer solution, 2 μL of enzyme reagent A and 10 μL of enzyme reagent B). Keep it on ice during use and store at -20°C for one day protected from light.

③ The preparation of enzyme B working solution：
Before testing, please prepare sufficient enzyme B working solution. For example, prepare 1005 μL of enzyme B working solution (mix well 1000 μL of buffer solution and 5 μL of enzyme reagent B). Keep it on ice during use and store at -20°C for one day protected from light.

④ The preparation of working solution：

Dissolve one vial of enzyme reagent C with 84 μL of double distilled water, mix well to get enzyme C working solution, which should be used up within same day.

Before testing, please prepare sufficient working solution. For example, prepare 2058 μL of working solution (mix well 2000 μL of buffer solution, 28 μL of enzyme C working solution and 30 μL of probe). Keep it on ice during use and used up within 30 min.

⑤ The preparation of 50 μmol/L standard solution：

Before testing, please prepare sufficient 50 μmol/L standard solution. For example, prepare 1000 μL of 50 μmol/L standard solution (mix well 50 μL of 1 mmol/L standard and 950 μL of double distilled water). The 50 μmol/L standard solution should be prepared on spot and used up within same day.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 50 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 35, 40 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**25**	**30**	**35**	**40**
50 μmol/L Standard (μL)	0	20	40	80	100	120	140	160
Double distilled water (μL)	200	180	160	120	100	80	60	40

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells in 200 μL normal saline (0.9% NaCl)(3 times).

③ Lyse 1×10^6 cells with 200 μL extraction solution. Place on the ice box and mix well every 5 min, lyse for 10 min.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	15-20
10% Mouse brain tissue homogenate	15-20
Human serum	80-100
Fetal bovine serum	80-100
Mouse plasma	80-100
Rat plasma	80-100
1×10^6 Jurkat cells	1
1×10^6 293T cells	1
1×10^6 HL-60 cells	1
1×10^6 Hela cells	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① For a small volume of enzyme reagent A, enzyme reagent B and enzyme reagent C, please centrifuge before use.

② The working solution should be prepared before use, and the placement time should not be too long, otherwise the background fluorescence value is too high, which will affect the experimental results.

③ When preparing the standard of different concentrations, it can be properly centrifuged and mixed. It should be observed that the standard should be a transparent liquid. If there is flocculation precipitation, it can be ultrasonic until the solution is completely dissolved.

④ After adding working solution, the plate can be slightly oscillated to make the reaction reagent mixed fully.

Operating steps

① Standard well: add 10 μL of standard with different concentrations into the well.
Sample well: add 10 μL of sample into the well.
Control well: add 10 μL of sample into the well.
② Add 80 μL of enzyme A working solution into standard and sample wells.
③ Add 80 μL of enzyme B working solution into control wells.
④ Add 100 μL of working solution into each well.
⑤ Mix fully with fluorescence microplate reader for 5s. Incubate at 37°C for 40 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum and plasma samples:

$$\text{SM content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue and cell samples:

$$\text{SM content (μmol/gprot)} = (\Delta F - b) \div a \div C_{pr} \times f$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{control}$.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15.00	27.00	32.00
%CV	5.1	4.9	4.9

Inter-assay Precision

Three mouse serum were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15.00	27.00	32.00
%CV	10.0	6.0	7.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	15	27	32
Observed Conc.(μmol/L)	15.3	28.1	32.0
Recovery rate (%)	102	104	100

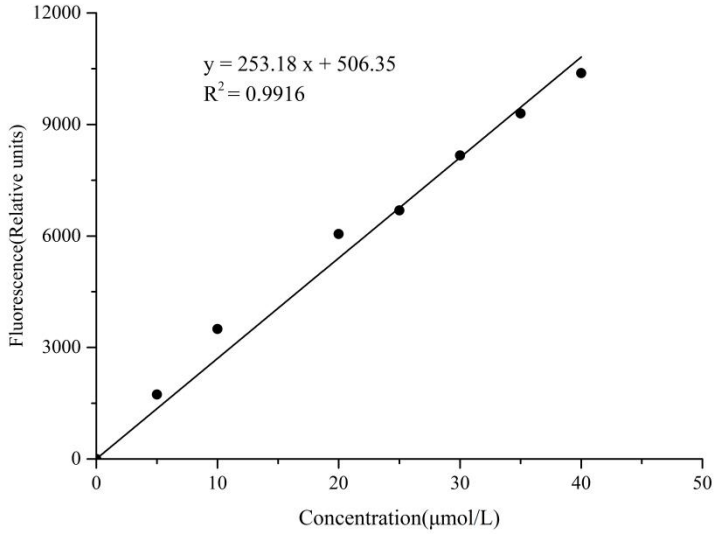
Sensitivity

The analytical sensitivity of the assay is 0.35 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	5	10	20	25	30	35	40
Fluorescence value	1051	2763	4431	7041	7567	9063	10193	11232
	1022	2778	4637	7139	7885	9348	10476	10476
Average fluorescence value	1037	2771	4534	7090	7726	9206	103349	11420
Absoluted fluorescence value	0	1734	3497	6054	6689	8169	9298	10384



Appendix Π Example Analysis

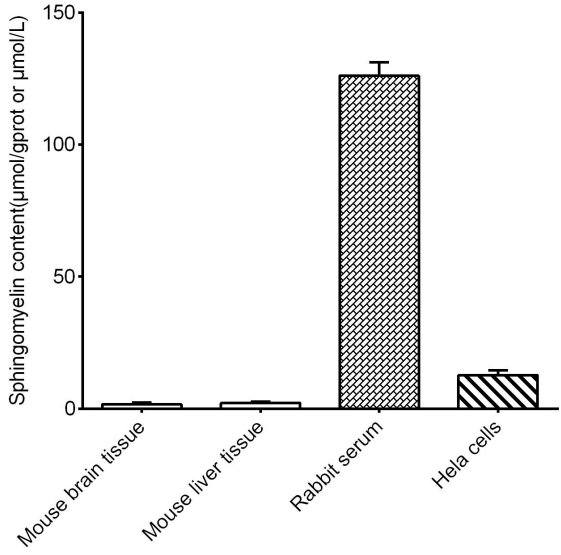
Example analysis：

Take 20 μL of 10% mouse brain tissue supernatant which dilute for 20 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 362.63 x + 370.99, the average fluorescence value of the sample well is 6002, the average fluorescence value of the control well is 5426, $\triangle F = F_{sample} - F_{control} = 6002 - 5426 = 576$, the concentration of protein is 6.78 gprot/L, and the calculation result is:

SM content (μmol/gprot) = (576 - 370.99) ÷ 362.63 × 20 ÷ 6.78 = 1.67 μmol/gprot

Detect 10% mouse brain tissue homogenate (the concentration of protein is 6.78 gprot/L, dilute for 20 times), 10% mouse liver tissue homogenate (the concentration of protein is 10.08 gprot/L, dilute for 20 times), rabbit serum (dilute for 100 times) and 1×10^6 Hela cells (the concentration of protein is 0.7 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.