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

Catalog No：E-BC-K841-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(405-415 nm)

Detection range：1.34-37.14 U/L

Elabscience® S-Adenosyl-Homocysteine Hydrolase (SAHH) Activity Colorimetric 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
Tel: 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.

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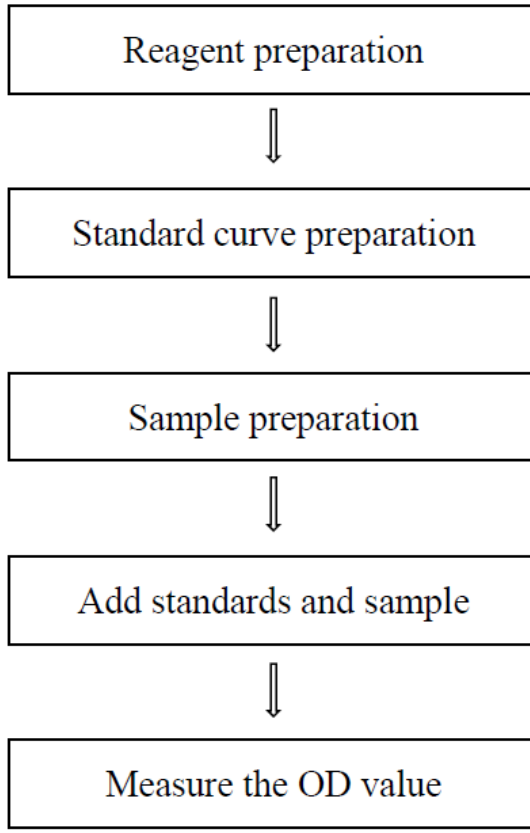
Assay summary



Intended use

This kit can be used to measure S-Adenosyl-Homocysteine Hydrolase (SAHH) activity in animal tissue and cell samples.

Detection principle

In mammalian cells, S-adenosine homocysteine hydrolase (SAHH) is the only hydrolase involved in the hydrolysis of S-adenosine homocysteine (SAH) to homocysteine (Hcy). Excessive SAHH activity can hydrolyze SAH to generate excessive Hcy, thereby causing geriatric diseases such as Alzheimer's disease, hypercysteinemia, and coronary heart disease. Inhibiting SAHH activity can effectively alleviate the hydrolysis of SAH.

The detection principle of this kit: The substrate is hydrolyzed by SAHH, and the product reacts with the chromogenic agent at room temperature to form a yellow colored product, which has the maximum absorption peak at 410 nm. The activity of SAHH can be calculated by detecting the change in absorbance at 410 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL×1 vial	-20℃, 12 months shading light
Reagent 2	Substrate	0.15 mL ×1 vial	-20℃, 12 months shading light
Reagent 3	Co-factor	1.8 mL ×1 vial	-20℃, 12 months shading light
Reagent 4	Chromogenic Agent	0.3 mL ×1 vial	-20℃, 12 months shading light
Reagent 5	10 mmol/L Standard Solution	0.8 mL ×1 vial	-20℃, 12 months shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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:

Microplate reader (405 - 415 nm, optimum wavelength: 410 nm), Incubator (37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25 ℃ before use.

② The preparation of substrate working solution:

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 90 μL of substrate working solution (mix well 10 μL of substrate and 80 μL of buffer solution). The substrate working solution should be prepared on spot and keep the solution on ice during use. Store at -20 ℃ for 3 days protected from light.

③ The preparation of Co-factor working solution:

For each well, prepare 100 μL of Co-factor working solution (mix well 20 μL of Co-factor and 80 μL of buffer solution). The Co-factor working solution should be prepared on spot and keep the solution on ice during use. Store at -20 ℃ for 3 days protected from light.

④ The preparation of chromogenic working solution：

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 100 μL of chromogenic working solution (mix well 10 μL of chromogenic agent and 90 μL of buffer solution). The chromogenic working solution should be prepared on spot and keep the solution on ice during use. Store at -20 ℃ for 3 days protected from light.

⑤ The preparation of 1 mmol/L standard solution：

Before testing, please prepare sufficient 1 mmol/L standard solution. For example, prepare 1000 μL of 1 mmol/L standard solution (mix well 100 μL of 10 mmol/L standard solution and 900 μL of buffer solution). The 1 mmol/L standard solution should be prepared on spot and keep the solution on ice during use. Store at -20 ℃ for 3 days.

⑥ The preparation of standard curve：

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

Dilute 1 mmol/L standard solution with extracting solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.3, 0.5, 0.7, 0.8, 0.9, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑧	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.3**	**0.5**	**0.7**	**0.8**	**0.9**	**1**
1 mmol/L standard (μL)	0	20	60	100	140	160	180	200
Buffer Solution (μL)	200	180	140	100	60	40	20	0

Sample preparation

① Sample preparation

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

③ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection, detect the prepared supernatant within 4 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell sample:

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

② Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

③ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection, detect the prepared supernatant within 4 h.

④ 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	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse leg muscle tissue homogenate	1
1×10^6 293T cells	1
1×10^6 Jurkat cells	1
1×10^6 Molt-4 cells	1
1×10^6 Hela cells	1
1×10^6 HL-60 cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standards with different concentrations into the standard wells.

Sample well: add 20 μL of sample into the sample wells.

Control well: add 20 μL of sample into the control wells.

② Standard well: Add 120 μL of buffer solution to the standard wells.

Control well: Add 20 μL of buffer solution to the control wells.

Sample well: Add 20 μL of substrate working solution into the sample wells.

③ Add 100 μL of Co-factor working solution to the sample wells and control wells.

④ Add 20 μL of chromogenic working solution to each well.

⑤ Mix fully for 5 s with microplate reader and incubate at 37℃ for 20 min. Measure the OD values of each well at 410 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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:

Tissue and cell sample:

Definition: The amount of 1 g tissue or cell protein that hydrolyze the substrate to produce 1 μmol Hcy in 1 min at 37℃ is defined as 1 unit.

$$\text{SAHH activity(U/gprot)} = (\Delta A_{410} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

[Note]

ΔA_{410}: $OD_{sample} - OD_{control}$, $\Delta A_{410} = A_{sample} - A_{control}$

T: Time for the sample to incubation reaction at 37 ℃, 20 min

1000: 1 mmol/L = 1000 μmol/L

f: Dilution factor of sample before test

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	10.00	20.00
%CV	3.4	2.7	1.8

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	10.00	20.00
%CV	7.8	6.8	3.7

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 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	5.00	10.00	20.00
Observed Conc.(U/L)	4.90	10.00	20.00
Recovery rate (%)	98.0	100.0	100.0

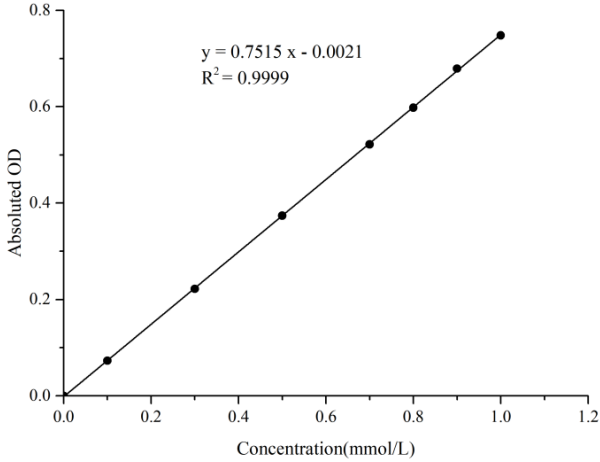
Sensitivity

The analytical sensitivity of the assay is 1.34 U/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 OD 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 (mmol/L)	**0**	**0.1**	**0.3**	**0.5**	**0.7**	**0.8**	**0.9**	**1.0**
OD	0.080	0.154	0.303	0.456	0.606	0.683	0.762	0.829
	0.081	0.152	0.301	0.452	0.599	0.674	0.757	0.828
Average OD	0.081	0.153	0.302	0.454	0.603	0.679	0.760	0.829
Absoluted OD	0	0.073	0.221	0.373	0.522	0.598	0.679	0.748



Appendix II Example Analysis

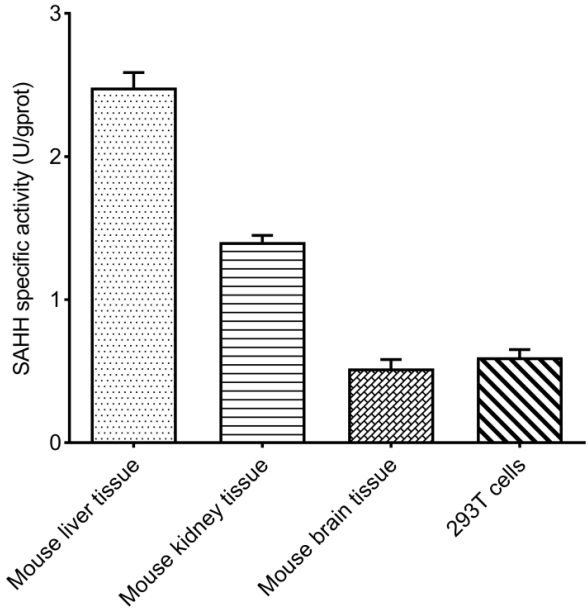
Example analysis:

Take 20 μL of 10% mouse kidney tissue homogenate into the wells and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.7515 x - 0.0021, the OD value of sample well is 0.963, the OD value of the control well is 0.798, 10% mouse kidney tissue homogenate (the concentration of protein is 7.82 gprot/L) and the calculation result is:

Δ SAHH activity(U/gprot) = (0.165 + 0.0021) ÷ 0.7515 ÷ 20 × 1000 ÷ 7.82 = 1.42 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 11.82 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 7.82 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein is 3.73 gprot/L), 1×10^6 293T cells (the concentration of protein is 0.55 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.