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

Catalog No：E-BC-K779-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader(550-560 nm)

Detection range：0.004-0.107 U/L

Elabscience® Acyl-CoA Synthetase (ACS) Activity Colorimetric Assay Kit

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

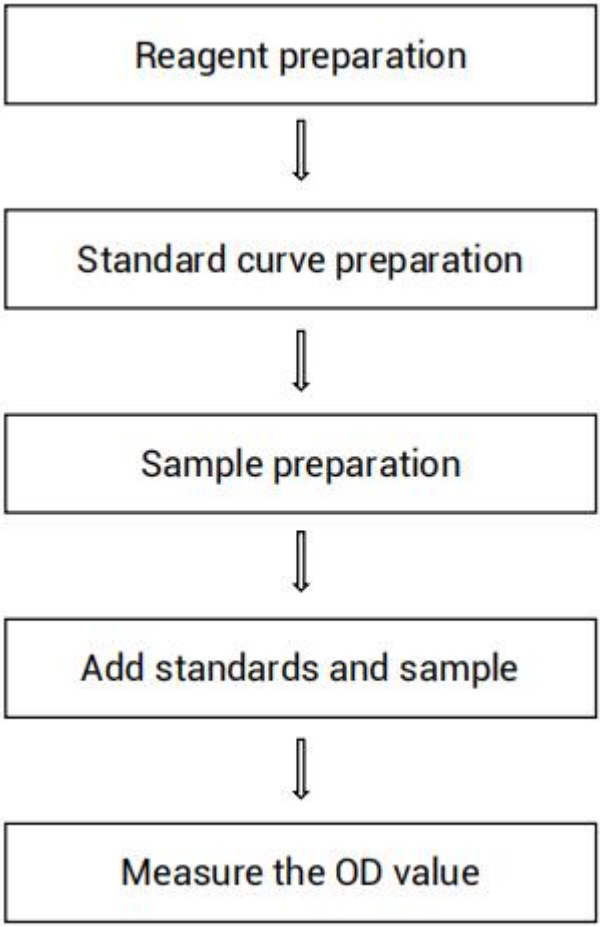
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
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 acyl-CoA synthetase (ACS) activity in serum, plasma, animal tissue, plant tissue and cell samples.

Detection principle

Acyl-CoA synthase (ACS) is an important enzyme that is widely present in living organisms and participates in the metabolic process of fatty acids. It is an enzyme necessary for lipid synthesis, fatty acid catabolism and membrane remodeling, and can also function as transcriptional activator, allosteric inhibitor or precursor of inflammatory mediators. In the medical field, mutations or abnormal expression of ACS may be associated with various diseases, such as diabetes and obesity.

The detection principle of this kit: The ACS catalyzes the substrate, and the generated product reacts with the chromogenic agent to produce a purple substance, which has the largest absorption peak at 555 nm. Therefore, the absorbance at 555 nm can be measured to calculate the amount of product generated, thereby calculating the activity of ACS.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Co-factor	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 3	Accelerant	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 4	Substrate	0.3 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Enzyme Reagent	3.6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent	3.6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 7	2 mmol/L Standard Solution	1.6 mL × 1 vial	-20℃, 12 months, shading light
	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:

Microplate reader (550-560 nm, optimum wavelength: 555 nm), Incubator

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of co-factor working solution:
Dissolve one vial of co-factor with 0.8 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of accelerant working solution:
Dissolve one vial of accelerant with 2.7 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 7 days protected from light.

④ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 60 μL of reaction working solution (mix well 10 μL of co-factor working solution and 50 μL of accelerant working solution). Keep it on ice during use protected from light. Store at -20°C for 2 days protected from light.

⑤ The preparation of substrate working solution:
The substrate was incubated at 37°C for 10 min. Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 100 μL of substrate working solution (mix well 5 μL of substrate and 95 μL of double distilled water). Keep it on ice during use protected from light. Store at -20°C for 2 days protected from light.

⑥ The preparation of chromogenic working solution:
For each well, prepare 60 μL of chromogenic working solution (mix well 30 μL of enzyme reagent and 30 μL of chromogenic agent). Keep it on ice during use protected from light. Store at -20°C for 2 days protected from light.

⑦ The preparation of standard curve：

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

Dilute 2 mmol/L standard with buffer solution to a serial concentration, the recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.8, 1, 1.2, 1.6, 2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.8**	**1**	**1.2**	**1.6**	**2**
2 mmol/L standard (μL)	0	20	40	80	100	120	160	200
Buffer solution (μL)	200	180	160	120	100	80	40	0

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 Homogenize 20 mg tissue in 180 μL buffer solution 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. The supernatant should be used within 4 h.

Cell (adherent or suspension) samples:

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 buffer solution 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. The supernatant should be used within 4 h.

② Dilution of sample

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

Sample type	Dilution factor
Human plasma	1
Mouse serum	1
10% Mouse liver tissue homogenate	1-4
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse leg muscle tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Corn seed tissue homogenate	1
1×10^6 Molt-4 cells	1

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

The key points of the assay

It is necessary to observe whether the substrate is clarified before use. If it is not completely clarified after 10 min at 37°C, the time can be extended appropriately.

Operating steps

① Standard well: Add 10 μL of standard with different concentrations into wells.
Sample well: Add 10 μL of sample into wells.
Control well: Add 10 μL of sample into wells.

② Add 100 μL of buffer solution into standard wells. Add 50 μL of reaction working solution into sample and control wells.

③ Add 50 μL of double distilled water into control wells. Add 50 μL of substrate working solution into sample wells.

④ Add 60 μL of chromogenic working solution into each well.

⑤ Mix fully with microplate reader for 5 s and Incubate at 37°C for 10 min protected from light. Measure the OD value of each well at 555 nm.

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:

1. Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that produce 1 mmol of H_2O_2 at 37 °C is defined as 1 unit.

$$\text{ACS activity (U/L)} = (\Delta A_{555} - b) \div a \div T \times f$$

2. Tissue samples:

Definition: The amount of enzyme in 1 kg tissue per 1 min t that produce 1 mmol of H_2O_2 at 37 °C is defined as 1 unit.

$$\text{ACS activity (U/kg wet weight)} = (\Delta A_{555} - b) \div a \div T \div m \times v \times f$$

3. Cell samples:

Definition: The amount of enzyme in 1×10^6 cells per 1 min that produce 1 μmol of H_2O_2 at 37 °C is defined as 1 unit.

$$\text{ACS activity (U/10^6)} = (\Delta A_{555} - b) \div a \div T \div n \times v \times f$$

[Note]

ΔA_{555}: $OD_{sample} - OD_{control}$.

T: Reaction time, 10 min.

m: The wet weight of sample, kg.

n: The number of cell sample/10^6.

V: The volume of buffer solution in the preparation step of sample, L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human plasma 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）	0.02	0.04	0.08
%CV	3.6	2.7	2.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.02	0.04	0.08
%CV	8.9	6.8	3.8

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 97.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.02	0.04	0.08
Observed Conc. (U/L)	0.020	0.040	0.076
Recovery rate (%)	98.0	100.0	95.0

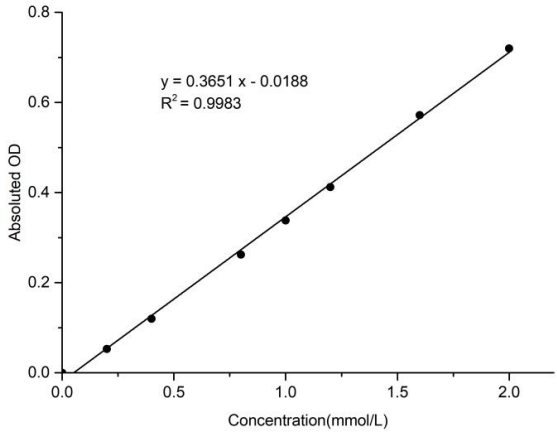
Sensitivity

The analytical sensitivity of the assay is 0.004 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**	**0.2**	**0.4**	**0.8**	**1**	**1.2**	**1.6**	**2**
OD value	0.058	0.108	0.176	0.318	0.391	0.470	0.627	0.778
	0.058	0.114	0.180	0.322	0.401	0.472	0.635	0.778
Average OD value	0.058	0.111	0.178	0.320	0.396	0.471	0.631	0.778
Absolute OD value	0	0.053	0.120	0.262	0.338	0.412	0.572	0.720



Appendix Π Example Analysis

Example analysis：

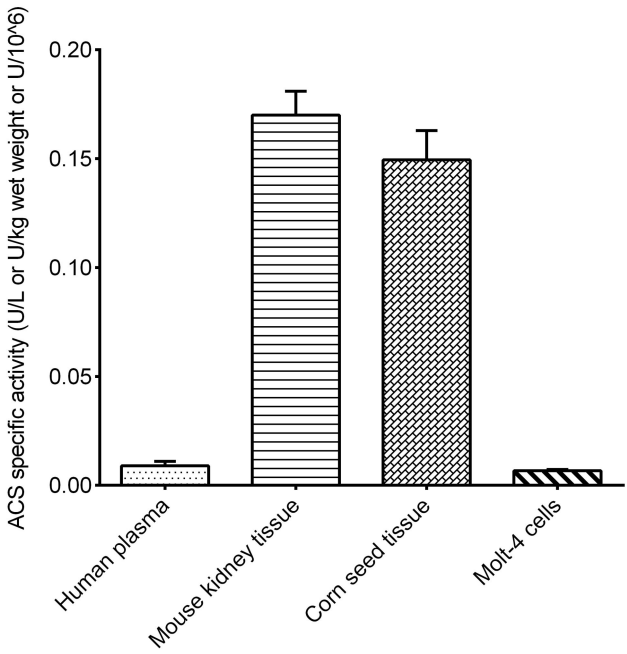
Take 10 μL of 10% mouse kidney tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.3651 x - 0.0188, the average OD value of the sample well is 0.136, the average OD value of the control well is 0.086, ΔA_{555} = OD_{sample} – $OD_{control}$ = 0.136 – 0.086 = 0.050, and the calculation result is:

ACS activity (U/kg wet weight) = (0.050 + 0.0188）÷ 0.3651 ÷ 10 ÷ 0.0001 × 0.0009

= 0.170 U/kg wet weight

Detect human plasma, 10% mouse kidney tissue homogenate, 10% corn seed tissue homogenate and 1×10^6 Molt-4 cells, 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.