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

Catalog No：E-BC-K794-M

Specification：48T (46 samples)/96T (94 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：0.44-16.62 U/L

Elabscience® Squalene Synthase (SQS) 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.

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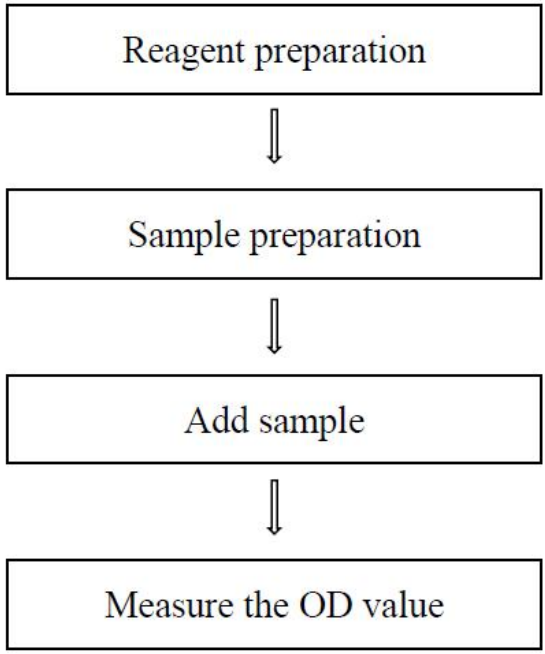
Assay summary



Intended use

This kit can measure squalene synthase (SQS) activity in serum, plasma, animal tissue and cell samples.

Detection principle

Squalene synthase (SQS) is involved in the isoprene biosynthesis pathway, and its catalytic action is the first rate-limiting step in sterol biosynthesis. Squalene synthetase can produce squalene under the co-action of cofactors. The principle of this kit is that the absorbance of cofactors can be measured at 340 nm, and the amount of cofactors consumed in the catalytic reaction is proportional to the activity of squalene synthase, and the activity of squalene synthase can be calculated according to the change of absorbance value per unit time.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months, shading light
Reagent 2	Buffer Solution	7 mL × 1 vial	13 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate A	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 4	Substrate B	0.12 mL × 1 vial	0.24 mL × 1 vial	-20°C, 12 months, shading light
	UV-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 (340 nm), Incubator(37℃)

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of substrate A working solution:
Dissolve one vial of substrate A with 3 mL of buffer solution, mix well to dissolve. Store at -20°C for 3 days protected from light.

③ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 500 μL of working solution (mix well 10 μL of substrate B and 490 μL of substrate A working solution). The working solution should be prepared on spot.

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 cold PBS (0.01 M, pH 7.4).

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

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

Cell (adherent or suspension) samples:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

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

② Dilution of sample

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1
1×10^6 Hela 60 cells	1
Rat plasma	1

Porcine plasma	1
Human plasma	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

The volume of substrate B is small, please centrifuge before use.

Operating steps

① Control well: Add 10 μL of double distilled water to each well.
Sample well: Add 10 μL of samples to each well.

② Add 100 μL of working solution to each well.

③ Measure the OD value of each well at 340 nm with microplate reader, as A_1.

④ Incubate at 37°C for 40 min, measure the OD value of each well at 340 nm with microplate reader, as A_2.

Calculation

The sample:

1. Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum (plasma) per 1 min that consume 1 μmol NADPH at 37°C is defined as 1 unit.

$$\text{SQS activity (U/L)} = \frac{\Delta A_{sample} - \Delta A_{control}}{\varepsilon \times d} \times \frac{V_1}{V_2} \div T \times f \times 1000$$

2. Tissue samples:

Definition: The amount of enzyme in 1 kg tissue per 1 min that consume 1 μmol NADPH at 37°C is defined as 1 unit.

$$\text{SQS activity (U/kg wet weight)} = \frac{\Delta A_{sample} - \Delta A_{control}}{\varepsilon \times d} \times \frac{V_1}{V_2} \div \frac{m}{V} \div T \times f \times 1000$$

3. Cell samples:

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

$$\text{SQS activity (U/1×10^6)} = \frac{\Delta A_{sample} - \Delta A_{control}}{\varepsilon \times d} \times \frac{V_1}{V_2} \div \frac{n}{V_3} \div T \times f$$

[Note]

ΔA_{sample}: Absolute OD value of the sample, $A_1 - A_2$.

$\Delta A_{control}$: Absolute OD value of the control, $A_1 - A_2$.

ε: The molar extinction coefficient, 6.22 L/mmol/cm.

d: Optical path of microplate wells, 0.5 cm.

V_1: The volume of reaction system, 110 μL.

V_2: The volume of sample, 10 μL.

m: The weight of tissue, g.

V: The volume of extraction solution in the preparation step of tissue, mL.

T: Reaction time, 40min.

f: Dilution factor of sample before test.

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

V_3: The volume of extraction solution in the preparation step of cell, mL.

1000: 1 mmol = 1000 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat serum 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)	2.50	3.90	1.60
%CV	1.9	5.0	3.2

Inter-assay Precision

Three rat serum 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)	2.50	3.90	1.60
%CV	2.1	7.2	3.2

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	2.5	3.9	1.6
Observed Conc. (U/L)	2.4	4.3	1.5
Recovery rate (%)	95	109	96

Sensitivity

The analytical sensitivity of the assay is 0.44 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.

Appendix II Example Analysis

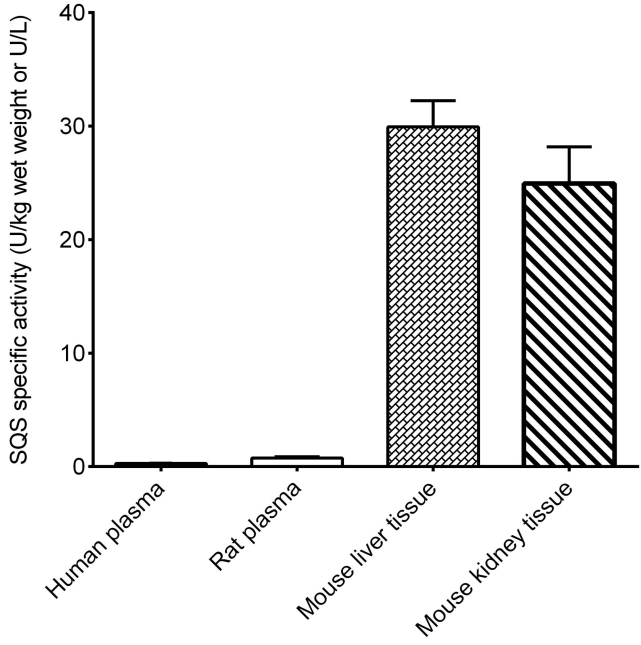
Example analysis:

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

The A_1 value of the sample is 0.599, the A_2 value of the sample is 0.555, ΔA_{sample} = 0.599 – 0.555 = 0.044; the A_1 value of the control is 0.425, the A_2 value of the control is 0.421, $\Delta A_{control}$ = 0.425 - 0.421 = 0.004, and the calculation result is:

SQS activity (U/kg wet weight) = (0.044 - 0.004) ÷ (6.22 × 0.5) × (110 ÷ 10) ÷ (0.1 ÷ 0.9) ÷ 40 × 1000 = 31.83 U/kg wet weight

Detect human plasma, rat plasma, 10% mouse liver tissue homogenate, 10% mouse kidney tissue homogenate, 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.