

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

Catalog No: E-BC-K1109-M

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

Measuring instrument: Microplate reader(340 nm)

Detection range: 0.75-75 U/L

Elabscience® Fatty Acid Synthase (FAS) 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

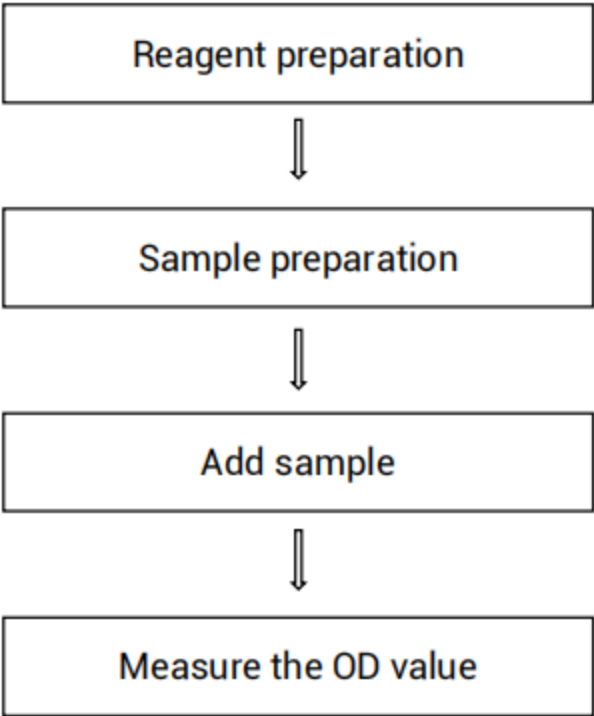
Website: www.elabscience.com

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

The kit is suitable for detecting the activity of fatty acid synthase (FAS) in serum, plasma, animal tissues plant tissues and cells.

Detection principle

Fatty Acid Synthase (FAS) and its synthesized fatty acid products modulate intracellular signaling pathways, thereby regulating critical biological processes such as cell proliferation, differentiation, and apoptosis.

FAS catalyzes substrate reactions that consume NADPH. Since NADPH exhibits a characteristic absorption peak at 340 nm, the activity of FAS can be determined by measuring the rate of decrease in the sample's OD value at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	55 mL × 2 vials	-20°C,12 months
Reagent 2	Buffer Solution	11 mL × 1 vial	22 mL × 1 vial	-20°C,12 months
Reagent 3	Substrate A	Powder × 1 vial	Powder × 2 vials	-20°C,12 months, shading light
Reagent 4	Substrate B	Powder × 1 vial	Powder × 2 vials	-20°C,12 months, shading light
Reagent 5	Cofactor	Powder × 1 vial	Powder × 2 vials	-20°C,12 months, shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplatereader (340 nm), Incubator

Reagent preparation

- ① Equilibrate all the reagents to 25°C before use.
- ② Substrate A Working Solution preparation:
Dissolve one vial of Substrate A with 0.4 mL of Buffer Solution. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.
- ③ Substrate B Working Solution preparation:
Dissolve one vial of Substrate B with 0.4 mL of Buffer Solution. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.
- ④ Cofactor Working Solution preparation:
Dissolve one vial of Cofactor with 0.4 mL of Buffer Solution. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.
- ⑤ Chromogenic Working Solution preparation:
Before testing, please prepare sufficient Chromogenic Working Solution according to the test wells. For example, prepare 1000 µL of Working Solution (mix well 850 µL of Buffer Solution, 50 µL of Substrate A Working Solution, 50 µL of Substrate B Working Solution and 50 µL of Cofactor Working Solution). The Chromogenic Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

Sample preparation

Serum and plasma: 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 μ L Extraction Solution with a dounce homogenizer at 4°C.
- ④ Centrifuge at 10000 $\times$ g for 10 min at 4°C. Collect supernatant and keep it on ice for detection and detect within 8 h.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M/E-BC-K168-M).

Cell sample:

- ① Harvest the number of cells needed for each assay (initial recommendation 1×10^7 cells). Wash cells with PBS (0.01 M, pH 7.4).
- ② Homogenize 1×10^6 cells in 200 μ L Extraction Solution with a dounce homogenizer.
- ③ Centrifuge at 10000 $\times$ g for 10 min at 4°C. Collect supernatant and keep it on ice for detection and detect within 8 h.
- ④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Dilution of sample

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

Sample type	Dilution factor
Human serum	1
10% Mouse kidney tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Potato tissue homogenization	1
10% Sweet potato tissue homogenization	1
1×10 ⁷ RAW 264.7 cells	1

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

Operating steps

- ① Control well: Add 20 μL of Extraction Solution to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

- ② Add 130 μL of Chromogenic Working Solution to each well.
- ③ Shake the microplate for 5 seconds to ensure complete mixing. Measure the OD value of each well at 340 nm, as A_1 . Incubate at 37°C for 20 min and measure the OD value of each well at 340 nm, as A_2 .

Calculation

The sample:

1. Serum and plasma samples:

$$\begin{aligned}\text{FAS activity} \\ (\text{U/L}) &= (\Delta A_{\text{sample}} - \Delta A_{\text{control}}) \times V_1 \div \epsilon \div d \div V_2 \div T \times 10^6 \times f \\ &= (\Delta A_{\text{sample}} - \Delta A_{\text{control}}) \times 150.72 \times f\end{aligned}$$

2. Tissue sample:

$$\begin{aligned}\text{FAS activity} \\ (\text{U/gprot}) &= (\Delta A_{\text{sample}} - \Delta A_{\text{control}}) \times V_1 \div \epsilon \div d \div V_2 \div T \div C_{\text{pr}} \times 10^6 \times f \\ &= (\Delta A_{\text{sample}} - \Delta A_{\text{control}}) \times 150.72 \div C_{\text{pr}} \times f\end{aligned}$$

[Note]

ΔA_{sample} : The OD value of the sample well changes., $A_2 - A_1$.

$\Delta A_{\text{control}}$: The OD value of the control well changes., $A_2 - A_1$.

ϵ : The molar extinction coefficient, $6.22 \times 10^3 \text{ L/mol/cm}$.

d : Optical path of microplate wells, 0.4 cm.

V_1 : The volume of reaction system, 0.15 mL.

V_2 : The volume of sample, 0.02 mL.

T : Reaction time, 20min.

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

10^6 : $1 \text{ mol} = 1 \times 10^6 \text{ } \mu\text{mol}$.

f : Dilution factor of sample before tested.

150.72: Simplified value.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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/gprot)	1.67	2.20	3.30
%CV	2.5	2.1	1.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/gprot)	1.990	3.790	5.730
%CV	6.5	8.5	8.9

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/gprot)	1.450	2.170	3.510
Observed Conc. (U/gprot)	1.400	2.180	3.630
Recovery rate (%)	97%	100%	103%

Sensitivity

The analytical sensitivity of the assay is 0.75 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 Π Example Analysis

Example analysis:

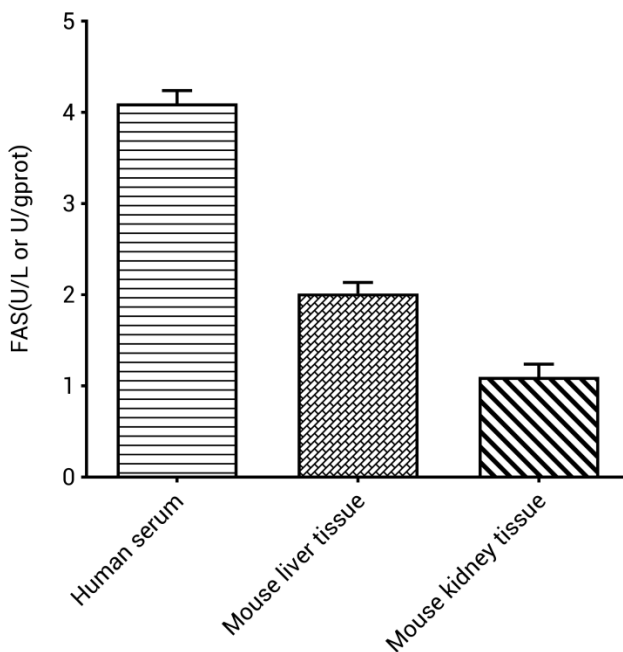
Take 20 μ L of 10% mouse liver tissue homogenate to the well of microplate.

Proceed according to the operating steps. The results are as follows:

The A_1 value of the sample is 0.853, the A_2 value of the sample is 0.680, $\Delta A_{\text{sample}} = 0.853 - 0.680 = 0.173$; the A_1 value of the control is 0.732, the A_2 value of the control is 0.718, $\Delta A_{\text{control}} = 0.732 - 0.718 = 0.014$, the concentration of protein is 12.40 gprot/L, and the calculation result is:

$$\text{FAS activity (U/gprot)} = (0.173 - 0.014) \times 150.72 \div 12.4 = 1.93 \text{ U/gprot}$$

Detect human serum, 10% mouse liver tissue homogenate supernatant (the concentration of protein is 12.40 gprot/L), 10% mouse kidney tissue homogenate supernatant (the concentration of protein is 6.68 gprot/L) 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.