

## Fatty Acid Synthase Assay Kit (Microanalysis)

### Description

Fatty Acid Synthase (FAS) is a key enzyme in fatty acid synthesis, catalyzing the formation of long-chain fatty acids from acetyl-CoA and malonyl-CoA. FAS is widely expressed in various tissues and cells, with particularly high expression in mammalian liver, kidney, brain, lung, mammary gland, and adipose tissue.

### Detection Principle

Fatty acid synthase catalyzes the conversion of acetyl-CoA, malonyl-CoA, and NADPH to long-chain fatty acids and NADP<sup>+</sup>. NADPH exhibits an absorption peak at 340 nm, whereas NADP<sup>+</sup> does not. FAS activity is calculated by measuring the rate of decrease in absorbance at 340 nm.

### Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
CB0071M-A	100 mL x 1	Store at -20°C; take out 1 day before use, thaw completely at 4°C and mix well before use
CB0071M-B	1 vial (powder) x 1	Store at 4°C; add 440 µL CB0071M-D before use and dissolve completely
CB0071M-C	1 vial (powder) x 1	Store at 4°C; add 440 µL CB0071M-D before use and dissolve completely
CB0071M-D	50 mL x 1	Store at 4 °C
CB0071M-E	1 vial (powder) x 1	Store at 4°C; add 840 µL CB0071M-D before use and dissolve completely

**Note:** Before formal measurement, be sure to select 2–3 samples with relatively large expected differences for a preliminary test.

### Instructions

#### I. Required Equipment & Materials:

Mortar, ice, benchtop centrifuge, UV spectrophotometer, micro quartz cuvette/96-well plate, adjustable pipettes, and distilled water.

#### II. Crude Enzyme Extraction:

##### 1. Tissue:

According to tissue weight (g), add CB0071M-A at a ratio of 1:5–10 (mL) (recommended: weigh about 0.1 g tissue and add 1 mL CB0071M-A), then homogenize in an ice bath. Centrifuge at 16,000 rpm for 40 min at 4°C, collect the supernatant, and keep it on ice for analysis.

##### 2. Bacteria or fungi:

According to the number of cells (10<sup>4</sup>), add CB0071M-A at a ratio of 500–1000:1 (mL) (recommended: add 1 mL CB0071M-A to 5 × 10<sup>6</sup> cells). Disrupt the cells by ultrasonication in an ice bath (power 300 W, sonicate for 3 s with 7 s intervals, total time 3 min). Then centrifuge at 16000 rpm for 40 min at 4°C, collect the supernatant, and keep it on ice for analysis.

##### 3. Serum or other liquid samples:

Measure directly.

### III. Assay Procedure

1. Preheat the spectrophotometer for at least 30 minutes, set the wavelength to 340 nm, and use distilled water to zero the instrument.
2. Preheat CB0071M-D in a 40°C water bath for 30 min.
3. Sample measurement (add the following reagents sequentially into a 1 mL quartz cuvette):

	Blank Tube (μL)	Sample Tube (μL)
Distilled Water	20	
CB0071M-B	4	
CB0071M-C	4	
CB0071M-D	164	
CB0071M-E	8	
Mix thoroughly, measure the absorbance at 340 nm at 30 s and 90 s, and record as A1 and A2, respectively. $\Delta A_{\text{blank}} = A1 - A2$ .		
Supernatant		20
CB0071M-B		4
CB0071M-C		4
CB0071M-D		164
CB0071M-E		8
Mix thoroughly, measure the absorbance at 340 nm at 30 s and 90 s, and record as A3 and A4, respectively. $\Delta A_{\text{sample}} = A3 - A4$ .		

**Note:** The blank tube only needs to be measured once.

### IV. Calculation of FAS Activity

#### 1. When using a micro quartz cuvette for measurement, the calculation formula is as follows:

(1) Based on protein concentration:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 μmol NADPH per minute per mg protein at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/\text{mg prot}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div (\text{Cpr} \times V_{\text{sample}}) \div T$$

$$= 1.61 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \text{Cpr}$$

(2) Based on sample mass:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 μmol NADPH per minute per g tissue at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/\text{g}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div (W \times V_{\text{sample}} \div V_{\text{total\_sample}}) \div T$$

$$= 1.61 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div W$$

(3) Based on cell number:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 μmol NADPH per minute per 10<sup>4</sup> cells at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/10^4 \text{ cell}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div (\text{cell number} \times V_{\text{sample}} \div V_{\text{total\_sample}}) \div T$$

$$= 1.61 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \text{cell number}$$

(4) Based on liquid volume:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 μmol NADPH per minute per mL sample at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/\text{mL}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div V_{\text{sample}} \div T$$

$$= 1.61 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}})$$

Note:

$\epsilon$ : Molar extinction coefficient of NADPH,  $6.22 \times 10^3$  L/mol/cm

d: Optical path length of cuvette, 1 cm

$V_{total}$ : Total reaction volume,  $200 \mu\text{L} = 2 \times 10^{-4}$  L

Cpr: Protein concentration of the supernatant (mg/mL)

W: Sample weight

$V_{sample}$ : Volume of supernatant added to the reaction system,  $20 \mu\text{L} = 0.02$  mL

$V_{total\_sample}$ : Volume of extraction solution, 1 mL

T: Reaction time, 1 min

**2. When using a 96-well plate, the calculation formula is as follows:**

(1) Based on protein concentration:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1  $\mu\text{mol}$  NADPH per minute per mg protein at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/\text{mg prot}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div (\text{Cpr} \times V_{\text{sample}}) \div T$$
$$= 3.22 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \text{Cpr}$$

(2) Based on sample mass:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1  $\mu\text{mol}$  NADPH per minute per g tissue at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/\text{g}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div (W \times V_{\text{sample}} \div V_{\text{total\_sample}}) \div T$$
$$= 3.22 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div W$$

(3) Based on cell number:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1  $\mu\text{mol}$  NADPH per minute per  $10^4$  cells at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/10^4 \text{ cell}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div (\text{cell number} \times V_{\text{sample}} \div V_{\text{total\_sample}}) \div T$$
$$= 3.22 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \text{cell number}$$

(4) Based on liquid volume:

Definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1  $\mu\text{mol}$  NADPH per minute per mL sample at 37°C.

$$\text{FAS } (\mu\text{mol}/\text{min}/\text{mL}) = [(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \div \epsilon \div d \times V_{\text{total}} \times 10^6] \div V_{\text{sample}} \div T$$
$$= 3.22 \times (\Delta A_{\text{sample}} - \Delta A_{\text{blank}})$$

Note:

$\epsilon$ : Molar extinction coefficient of NADPH,  $6.22 \times 10^3$  L/mol/cm

d: Optical path length of 96-well plate, 0.5 cm

$V_{total}$ : Total reaction volume,  $200 \mu\text{L} = 2 \times 10^{-4}$  L

Cpr: Protein concentration of the supernatant (mg/mL)

W: Sample weight

$V_{sample}$ : Volume of supernatant added to the reaction system,  $20 \mu\text{L} = 0.02$  mL

$V_{total\_sample}$ : Volume of extraction solution, 1 mL

T: Reaction time, 1 min

**Precautions**

1. The prepared reagents should be stored at 4°C and used within 3 days.
2. For protein concentration determination, it is recommended to use the BCA Protein Quantification Kit (C0050) produced by TargetMol.

3. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
4. Please wear a lab coat and disposable gloves.

### TargetMol US

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