Instruction Manual

Fatty Acid Synthase Assay Kit (Spectrophotometry)

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 acetyl-CoA, malonyl-CoA, and NADPH to generate long-chain fatty acids and $NADP^+$. NADPH has an absorption peak at 340 nm, whereas $NADP^+$ does not. The FAS activity is calculated by measuring the rate of decrease in absorbance at 340 nm.

Packing

Taking 50T/48S packing for example:

Components	Packing	Storage
CB0071S-A	50 mL x 1	Store at -20°C; take out 1 day before use, thaw completely at 4°C and mix well before use
CB0071S-B	1 vial (powder) x 1	Store at 4°C; before use, add 1100 μL of CB0071S-D and dissolve completely
CB0071S-C	1 vial (powder) x 1	Store at 4°C; before use, add 1100 μL of CB0071S-D and dissolve completely
CB0071S-D	50 mL×1	Store at 4 °C
CB0071S-E	1 vial (powder) x 1	Store at 4°C; before use, add 2100 μL of CB0071S-D 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, 1 mL quartz cuvette, adjustable pipettes, and distilled water.

II. Crude Enzyme Extraction:

1. Tissue:

According to tissue weight (g), add CB0071S-A at a ratio of 1:5–10 (mL) (recommended: weigh about 0.1 g tissue and add 1 mL CB0071S-A), then homogenize in an ice bath. Centrifuge at 16000 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 (10^4), add CB0071S-A at a ratio of 500–1000:1 (mL) (recommended: add 1 mL CB0071S-A to 5×10^6 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 30 min, set the wavelength to 340 nm, and use distilled water to zero the instrument.
2. Preheat CB0071S-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	100	
CB0071S-B	20	
CB0071S-C	20	
CB0071S-D	820	
CB0071S-E	40	
Mix thoroughly, measure the absorbance at 340 nm, and record the absorbance at 30 s and 90 s as A1 and A2, respectively. ΔA blank = A1 − A2.		
Supernatant		100
CB0071S-B		20
CB0071S-C		20
CB0071S-D		820
CB0071S-E		40
Mix thoroughly, measure the absorbance at 340 nm, and record the absorbance at 30 s and 90 s as A3 and A4, respectively. ΔA sample = A3 − A4.		

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

IV. Calculation of FAS Activity

(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.

FAS (μmol/min/mg prot) = $[(\Delta A_sample - \Delta A_blank) \div \varepsilon \div d \times V_total \times 10^6] \div (Cpr \times V_sample) \div T$

$= 1.61 \times (\Delta A_sample - \Delta A_blank) \div Cpr$

(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.

FAS (μmol/min/g) = $[(\Delta A_sample - \Delta A_blank) \div \varepsilon \div d \times V_total \times 10^6] \div (W \times V_sample \div V_total_sample) \div T$

$= 1.61 \times (\Delta A_sample - \Delta A_blank) \div W$

(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 10^4 cells at 37°C.

FAS (μmol/min/10^4 cell) = $[(\Delta A_sample - \Delta A_blank) \div \varepsilon \div d \times V_total \times 10^6] \div (\text{cell number} \times V_sample \div V_total_sample) \div T$

$= 1.61 \times (\Delta A_sample - \Delta A_blank) \div \text{cell number}$

(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.

FAS (μmol/min/mL) = $[(\Delta A_sample - \Delta A_blank) \div \varepsilon \div d \times V_total \times 10^6] \div V_sample \div T$

$= 1.61 \times (\Delta A_sample - \Delta A_blank)$

Note:

ε: Molar extinction coefficient of NADPH, 6.22×10^3 L/mol/cm

d: Optical path length of cuvette, 1 cm

V_{total}: Total reaction volume, 1000 μL = 0.001 L

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

W: Sample weight (g)

V_{sample}: Volume of supernatant added to the reaction system, 100 μL = 0.1 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.

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