EZMeta™ Fatty Acid Synthetase (FAS) Colorimetric Activity Kit

Cat #: D-AKC2240

Size: 48T / 96T

Storage: Stored at -20℃ for 6 months

Product Information

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria

Assay Principle

Fatty Acid Synthetase (FAS) is A key enzyme in fatty acid synthesis, catalyzing acetyl-coA and malonyl-coA to produce long chain fatty acids. FAS is widely expressed in various tissues and cells, and is abundant in liver, kidney, brain, lung, mammary gland and adipose tissue of mammals. EZMeta™ Fatty Acid Synthetase (FAS) Colorimetric Activity Kit provides a convenient tool for detection of FAS activity. The principle is that FAS catalyzes the production of long chain fatty acids and NADP+ from acetyl CoA, malonyl CoA and NADPH. NADPH has a characteristic absorption peak at 340 nm, while NADP+ does not. The enzyme activity of FAS was calculated by detecting the rate of decrease in absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	-20°C
Assay Buffer	10 mL	20 mL	4°C
NADPH	1	1	-20°C
Acetyl CoA	1	1	-20°C
Malonyl CoA	1	1	-20°C

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm

·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

·Ice maker, refrigerated centrifuge, incubator

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Take out 1d before use, defrost at 4°C and mix thoroughly. Equilibrate to room temperature. Store at -20°C.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

NADPH: Add 1.64 mL Assay Buffer for 96 T or 0.82 mL Assay Buffer for 48 T to dissolve before use. This solution can be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

Acetyl CoA: Add 0.44 mL Assay Buffer for 96 T or 0.22 mL Assay Buffer for 48 T to dissolve before use. This solution can be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

Malonyl CoA: Add 0.84 mL Assay Buffer for 96 T or 0.42 mL Assay Buffer for 48 T to dissolve before use. This solution can be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

Working Reagent: Prepare 180 µL Work Reagent for one well, add 16 µL of dissolved NADPH, 4 µL of dissolved Acetyl CoA, 8 µL of dissolved Malonyl CoA and 152 µL Assay Buffer. Prepare Working Reagent before use and depend on your need.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 12,000 g for 40 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 40 min at 4°C. Use supernatant for

assay, and place it on ice to be tested.

3. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 40 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

4. Serum, plasma, or other liquid samples: Tested directly.

Note: For animal tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. It will be better to quantify the total protein with EZMeta™ Fatty Acid Synthetase (FAS) Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Preheat the incubator to 37°C. Working Reagent is placed in incubator to preheat for more than 15 min.

3. Add 20 µL of sample, 180 µL of Working Reagent to the 96-well UV plate or microquartz cuvette, then tap the plate and mix well, quickly. Measure absorbance value at 340 nm. The 10 s absorbance value is recorded as A_1, and the 70 s absorbance value is recorded as A_2, calculate $\Delta A_{Test}=A_1-A_2$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.0005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.4, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculated by protein concentration

Unit definition: 1 nmol NADPH oxidated per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

FAS (U/mg prot)=(ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷(Cpr ×V_{Sample})÷T×n=**3,216×ΔA_{Test}÷Cpr×n**

2. Calculated by fresh weight of samples

Unit definition: 1 nmol NADPH oxidated per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

FAS (U/g)= (ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷(W ×V_{Sample}÷$V_{Sample\ Total}$)÷T×n=**3,216×ΔA_{Test}÷W×n**

3. Calculated the activity of FAS by cells or bacteria number

Unit definition: 1 nmol NADPH oxidated per min in 10^4 cells or bacteria reaction system is defined as a unit of enzyme activity.

FAS (U/10^4)=(ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷(total number of cells or bacteria×V_{Sample}÷$V_{Sample\ Total}$)÷T×n=3216×ΔA_{Test}÷500×n **=6.432×ΔA_{Test}×n**

4. Calculate the activity of FAS in liquid sample

Unit definition: 1 nmol NADPH oxidated per min in 1mL liquid sample reaction system is defined as a unit of enzyme activity.

FAS (U/mL)=(ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷V_{Sample}÷T×n=**3,216×ΔA_{Test}×n**

Where: ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; $V_{Reaction\ Total}$: total reaction volume, 200 μL=2×10^{-4} L; 10^9: 1 mol=1×10^9 nmol； Cpr: sample protein concentration, mg/mL; V_{sample}: sample volume added, 0.02 mL; T: reaction time, 1 min; n: dilution factor; W: sample weight, g; $V_{Sample\ Total}$: Extraction Buffer volume added, 1 mL; 500: Total number of cells or bacteria, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

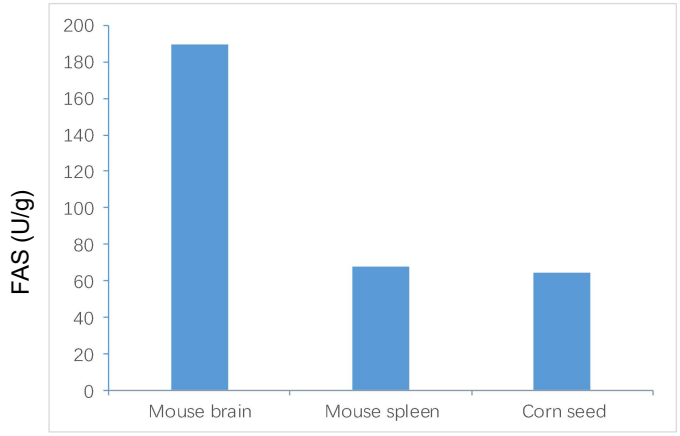

Figure 1. FAS activity in mouse brain, mouse spleen and corn seed respectively. Assays were performed following kit protocol.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.