Fatty Acid Synthase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Ultraviolet spectrophotometer/Microplate reader

Catalog Number: NA0583

Size: 100T/96S

Components:

Reagent	Size	Storage
Extract solution	Solution 110 mL×1	4°C
Reagent I	Powder×2	-20°C
Reagent II	Powder×2	-20°C
Reagent III	Solution 20 mL×1	4°C
Reagent IV	Powder×2	-20°C

Solution preparation:

1. Reagent I: Add 1 mL Reagent III before use. Mix thoroughly. Unused reagents should be store at -20°C for two weeks. Avoid repeated freezing and thawing.

2. Reagent II: Add 1 mL Reagent III before use. Mix thoroughly. Unused reagents should be store at -20°C for two weeks. Avoid repeated freezing and thawing.

3. Reagent III: Add 0.5 mL Reagent III before use. Mix thoroughly. Unused reagents should be store at -20°C for two weeks.

Product Description：

Fatty acid synthase (FAS) is an important enzyme in the synthesis of long-chain saturated fatty acids. It can catalyze malonyl coenzyme A, acetyl coenzyme A and NADPH to produce long chain fatty acids and $NADP^+$. NADPH has a characteristic absorption peak at 340nm. The activity of FAS can be calculated by measuring the decreasing rate of absorbance at 340nm.

Reagents and Equipment Required but Not Provided：

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, pipette, micro quartz cuvette/96 well UV flat -bottom plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

1. Bacteria or cells: According to the ratio of cells (10^4): Extract solution (mL) =500~1000:1. It is suggested to collect 5 million of cells and add 1 mL of Extract solution. Breaking cells on ice with ultrasonic wave (power 300W, ultrasonic wave 3 seconds, interval 9 seconds, total time 5 minutes) Centrifuge at 12 000 g, 4°C for 20 min. Take the supernatant for test.

2. Tissue: According to the ratio of tissue weight (g): Extract solution (mL) =1:5~10. It is suggested to weigh about 0.1 g of tissue and add 1 mL of Extract solution. Homogenize on ice. Centrifuge at 12 000 g,

4°C for 20 min. Take the supernatant for test.

3. Serum (plasma) and other liquid samples: direct determination.

II. Determination procedure：

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 min, adjust wavelength to 340 nm, set zero with distilled water.

2. Preheat the Reagent III at 37°C(mammal) or 25°C(other species) for 30 min.

3. Blank tube: Add 20 μL distilled water, 16 μL Reagent I, 16 μL Reagent II, 140 μL Reagent III and 8 μL Reagent IV in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 15s A1 and 1 min 15s A2 at 340 nm. Calculation ΔA_B= A1-A2.

4. Test tube: Add 20 μL supernatant, 16 μL Reagent I, 16 μL Reagent II, 140 μL Reagent III and 8 μL Reagent IV in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 15s A3 and 1 min 15s A4 at 340 nm. Calculation ΔA_T= A3-A4.

5. The blank tube only needs to be tested for 1-2 times. If the number of samples is too much, reagents one to four can be mixed according to the above ratio to prepare a working solution for measurement.

III. Calculations：

a. Micro quartz cuvette

1. Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADPH in the reaction system per minute every milligram protein.

FAS (U/mg prot) $=(\Delta A_T-\Delta A_B)\div (\varepsilon\times d) \times V_R\times10^9\div (V_S\times Cpr) \div T\times F=1608\times\Delta A\div Cpr\times F$

2. Calculate by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADPH in the reaction system per minute every gram tissue.

FAS (U/g weight) $=(\Delta A_T-\Delta A_B)\div (\varepsilon\times d) \times V_R\times10^9\div (W\times V_S\times V_E) \div T\times F=1608\times\Delta A\div W\times F$

3. Calculate by the amount of cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADPH in the reaction system per minute every 10^4 cell.

FAS (U/10^4 cell) $=(\Delta A_T-\Delta A_B)\div (\varepsilon\times d) \times V_R\times10^9\div (cell\times V_S\times V_E) \div T\times F=1608\times\Delta A\div cell\times F$

4. Calculate by the volume of liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADPH in the reaction system per minute every milliliter liquid.

FAS (U/mL) $=(\Delta A_T-\Delta A_B)\div (\varepsilon\times d) \times V_R\times10^9\div V_S\div T\times F=1608\times\Delta A\times F$

V_S： Add sample volume,0.02 mL；

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

d： Optical path of cuvette, 1 cm；

V_R: Total reaction volume, 200 μL=2×10^{-4}L；

V_E: Extract solution volume, 1 mL；

T: Reaction time, 1 min；

Cpr: Protein concentration of sample, mg/mL；

W: Sample weight, g;

F: Dilution ratio.

b.   96 well UV flat -bottom plate

The optical path d-1 cm in the above formula is changed to d-0.6 cm.

Note:

1. There is BSA (about 2mg/mL) in the Extract solution. When determining the protein concentration in the supernatant, the protein concentration in the Extract solution should be subtracted.

2. If the measured absorbance value A>1.5 or ΔA>0.5, it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

Experimental example

1.   Take 0.1 g of mouse liver. Add 1 mL of Extract solution. Homogenize on ice. Centrifuge at 12 000 g, 4°C for 20 min. Take the supernatant for test. Following the measurement procedure. Calculate ΔA_B=A1-A2=0.5653-0.5593=0.0060, ΔA_T=A3-A4=1.1783-1.0945=0.0838. Calculate the activity of FAS in mouse liver according to the formula:

FAS (U/g weight) =$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times VR\times10^9\div(W\times V_S\times V_E)\div T\times F$

$\qquad$ =$1608\times\Delta A\div W\times F$=1251.024 U/g weight.

2.   Take 0.1 g of mouse lung. Add 1 mL of Extract solution. Homogenize on ice. Centrifuge at 12 000 g, 4°C for 20 min. Take the supernatant for test. Following the measurement procedure. Calculate ΔA_B=A1-A2=0.5653-0.5593=0.0060, ΔA_T=A3-A4=1.3123-1.2769=0.0354. Calculate the activity of FAS in mouse lung according to the formula:

FAS (U/g weight) =$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times VR\times10^9\div(W\times V_S\times V_E)\div T\times F$

$\qquad$ =$1608\times\Delta A\div W\times F$=472.752 U/g weight.

Reference

[1] Robinson J D, Bradley R M, Brady R O. Biosynthesis of Fatty Acids[J]. Journal of Biological Chemistry, 1960, 238(2).

[2] Tcl B. Purification and crystallization of rat liver fatty acid synthetase[J]. Archives of Biochemistry & Biophysics, 1981, 209(2):613-619.

Related products

NA0701/NA0460   Lipase (LPS) Activity Assay Kit

NA0790/NA0549   Alcohol Dehydrogenase (ADH) Activity Assay Kit

NA0822/NA0580   Free fatty Acids (FFA) Content Assay Kit