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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: AK0288

Size: 100T/96S

Components:

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

Solution preparation:

1. Reagent I: Add 1 mL Reagent III before use. Mix thoroughly. Unused reagents should be store at -20℃ 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℃ 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℃ 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℃ 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℃ 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℃(mammal) or 25℃(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℃ 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) =$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times VR\times 10^9\div(W\times V_S\times V_E)\div T\times F$

=1608×ΔA÷W×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℃ 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) =$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times VR\times 10^9\div(W\times V_S\times V_E)\div T\times F$

=1608×ΔA÷W×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.

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AK0262/AK0261	Alcohol Dehydrogenase (ADH) Activity Assay Kit
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