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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

Catalog Number: AK0289

Size: 50T/48S

Components:

Reagent	Size	Storage
Extract solution	Solution 60 mL×1	4℃
Reagent I	Powder×2	-20℃
Reagent II	Powder×2	-20℃
Reagent III	Solution 55 mL×1	4℃
Reagent Ⅳ	Powder×2	-20℃

Solution preparation:

1. Reagent I: Add 2.5 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 2.5 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 1.25 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, desk centrifuge, pipette, water bath/ incubator, 1 mL quartz cuvette, 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 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 15 min.

3. Blank tube: Add 100 μL distilled water, 80 μL Reagent I, 80 μL Reagent II, 700 μL Reagent III and 40 μL Reagent Ⅳ in the 1 mL quartz cuvette. 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 100 μL supernatant, 80 μL Reagent I, 80 μL Reagent II, 700 μL Reagent III and 40 μL Reagent Ⅳ in the 1 mL quartz cuvette. 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：

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. 1 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, 1000 μL=1×10^{-3}L;

T: Reaction time, 1 min;

Cpr: Protein concentration of sample, mg/mL;

W: Sample weight, g;

F: Dilution ratio.

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>1.2 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 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=0.533-0.532=0.001, ΔA_T=A3-A4=1.221- 1. 195=0.026. 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=402 U/g weight.

2. 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=0.533-0.532=0.001, ΔA_T=A3-A4=1. 194- 1. 127=0.067. 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=1061.280 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. Purificati

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

Related products

AK0384/AK0383	Lipase (LPS) Activity Assay Kit
AK0262/AK0261	Alcohol Dehydrogenase (ADH) Activity Assay Kit
AK0536/AK0535	Free fatty Acids (FFA) Content Assay Kit