Fatty Acid Synthase Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0555

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 110 mL×1	2-8℃
Reagent I	Powder×2	-20℃
Reagent II	Powder×2	-20℃
Reagent III	Solution 20 mL×1	2-8℃
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 IV: Add 0.5 mL Reagent III before use. Mix thoroughly. Unused reagents should be store at -20℃ for two weeks. Avoid repeated freezing and thawing.

Product Description：

Fatty Acid Synthetase (FAS) is a rate limiting enzyme that plays an important role in the regeneration of fatty acids. It catalyzes the production of long-chain fatty acids and $NADP^+$ from acetyl CoA, acetyl CoA, and NADPH, with NADPH exhibiting a characteristic absorption peak at 340nm. By detecting the rate of decrease in absorbance under 340nm conditions, FAS activity can be calculated.

Reagents and Equipment Required but Not Provided：

Ultraviolet spectrophotometer/microplate reader, water-bath/constant temperature incubator, desk centrifuge, adjustable pipette, micro quartz cuvette/96 well UV plate, mortar/homogenizer/cell ultrasonic crusher, 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 3s, interval 9s, total time 5 minutes).

Centrifuge at 12000×g,

4℃ for 20min. Take the supernatant, placed on ice for testing.

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 12000 ×g, 4℃ for 20 min. Take the supernatant, placed on ice for testing.

3. Serum (plasma) and other liquid samples: direct determination. (If the solution is turbid, centrifuge to take the supernatant and then measure).

II. Determination procedure：

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

2. Preheat the Reagent III at 37℃ for 15 min.

3. Operation table (Add the following reagents to a 96 well UV plate or micro quartz cuvette):

Reagent name	Test tube (T)	Blank tube (B)
Sample	20	-
Distilled water	-	20
Reagent I	16	16
Reagent II	16	16
Reagent III	140	140
Reagent IV	8	8

Mix them immediately and time them. Record the absorbance value at 15s $A1_T$ ($A1_B$) and 1 min 15s $A2_T$ ($A2_B$) at 340 nm. Calculation $\Delta A = (A1_T - A2_T) - (A1_B - A2_B)$.

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

III. Calculations：

a. Calculation formula for determination using 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) $=\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (Vs \times Cpr) \div T \times F$

$=1607.7 \times \Delta A \div Cpr \times F$

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) $=\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (W \times Vs \div Ve) \div T \times F$

$=1607.7 \times \Delta A \div W \times F$

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) =ΔA÷ (ε×d) ×Vrv×10^9÷ (N×Vs÷Ve) ÷T×F
=1607.7×ΔA÷N×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) =ΔA÷ (ε×d) ×Vrv×10^9÷Vs÷T×F
=1607.7×ΔA×F

Vs：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；

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

Ve: 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;

N: Number of cells, ×10^4;

F: Dilution ratio.

b. Calculation formula for determination using 96 well UV plate

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) =ΔA÷ (ε×d) ×Vrv×10^9÷ (Vs×Cpr) ÷T×F
=2679.5×ΔA÷Cpr×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) =ΔA÷ (ε×d) ×Vrv×10^9÷ (W×Vs÷Ve) ÷T×F
=2679.5×ΔA÷W×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) =ΔA÷ (ε×d) ×Vrv×10^9÷ (N×Vs÷Ve) ÷T×F
=2679.5×ΔA÷N×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) =ΔA÷ (ε×d) ×Vrv×10^9÷Vs÷T×F
=2679.5×ΔA×F

Vs：Add sample volume, 0.1 mL；
ε：Micromolar extinction coefficient of NADPH, 6.22×10^3 L/mol/cm；
d：Optical path of 96 well UV plate, 0.6 cm；
Vrv: Total reaction volume, 200 μL=2×10^{-4}L;
Ve: 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;
N: Number of cells, ×10^4;
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>1.5 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) =1607.7×ΔA÷W×F=1251 U/g weight.

Recent Product Citations：

[1] Huang X, Pan L, Zuo Z, Li M, Zeng L, Li R, Ye Y, Zhang J, Wu G, Bai R, Zhuang L, Wei L, Zheng Y, Su J, Deng J, Deng S, Zhang S, Zhu S, Che X, Wang C, Wu C, Chen R, Lin D, Zheng J. LINC00842 inactivates transcription co-regulator PGC-1 伪 to promote pancreatic cancer malignancy through metabolic remodelling. Nat Commun. 2021 Jun 22;12(1):3830. doi: 10.1038/s41467-021-23904-4. PMID: 34158490; PMCID: PMC8219694.

[2] Meng X, Jayasundara N, Zhang J, Ren X, Gao B, Li J, Liu P. Integrated physiological, transcriptome and metabolome analyses of the hepatopancreas of the female swimming crab

Portunus trituberculatus under ammonia exposure. Ecotoxicol Environ Saf. 2021 Nov 25;228:113026. doi: 10.1016/j.ecoenv.2021.113026. Epub ahead of print. PMID: 34839137.

[3] Yang L, Zhao M, Liu M, Zhang W, Zhi S, Qu L, Xiong J, Wang L, Qin C, Nie G. Effects of Genistein on Lipid Metabolism, Antioxidant Activity, and Immunity of Common Carp (Cyprinus carpio L.) Fed with High-Carbohydrate and High-Fat Diets. Aquac Nutr. 2023 Mar 31;2023:9555855. doi: 10.1155/2023/9555855. PMID: 37034827; PMCID: PMC10081910.

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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