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Fatty Acid Synthase (FAS) 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
Catalog Number: AK0289-50T-48S
Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract Solution	Liquid 60 mL×1	2-8℃
Reagent I	Powder×2	-20℃
Reagent II	Powder×2	-20℃
Reagent III	Liquid 55 mL×1	2-8℃
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 IV: Add 1.25 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, water-bath/constant temperature incubator, desk centrifuge, adjustable pipette, 1 ml quartz cuvette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 test.

2. Tissue: According to the ratio of tissue mass (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 test.

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 the ultraviolet spectrophotometer for 30 min, adjust wavelength to 340 nm, set zero with distilled water.

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

3. Operation table (Add the following reagents to a 1mL quartz cuvette):

Reagent name	Test tube (T)	Blank tube (B)
Sample	100	-
Distilled water	-	100
Reagent I	80	80
Reagent II	80	80
Reagent III	700	700
Reagent IV	40	40

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:

1. Calculate by sample protein concentration

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

FAS activity(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 mass

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

FAS activity(U/g mass) $=\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (W \times Vs \div Ve) \div T \times F = 1607.7 \times \Delta A \div W \times F$

3. Calculate by the amount of cells

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

FAS activity($U/10^4$ cell) $=\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (N \times Vs \div Ve) \div T \times F = 1607.7 \times \Delta A \div N \times F$

4. Calculate by the volume of liquid

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

FAS activity(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, 1000 μL= 1×10^{-3}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 mass, g;
10^9: Reduction coefficient, 1mol=10^9nmol;

N: Number of cells, count by 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>1.2 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 12000 ×g, 4℃ for 20 min. Take the supernatant for test. Following the measurement procedure. Calculate $\Delta A_B=A1_B-A2_B=0.533-0.532=0.001$, $\Delta A_T=A1_T-A2_T=1.221-1.195=0.026$. Calculate the activity of FAS according to the formula:

FAS activity(U/g mass) =1607.7×ΔA÷W×F=418 U/g mass.

Recent Product Citations:

[1] Gao R, Li Y, Xu Z, Zhang F, Xu J, Hu Y, Yin J, Yang K, Sun L, Wang Q, He X, Huang K. Mitochondrial pyruvate carrier 1 regulates fatty acid synthase lactylation and mediates treatment of nonalcoholic fatty liver disease. Hepatology. 2023 Dec 1;78(6):1800-1815. doi: 10.1097/HEP.0000000000000279. Epub 2023 Jan 19. PMID: 36651176.

[2] Qu H, Shan K, Tang C, Cui G, Fu G, Qi Y, Cui J, Li J, Wang R, Feng N, Chen YQ. A novel small-molecule fatty acid synthase inhibitor with antitumor activity by cell cycle arrest and cell

division inhibition. Eur J Med Chem. 2021 Jul 5;219:113407. doi: 10.1016/j.ejmech.2021.113407. Epub 2021 Apr 20. PMID: 33901805.

[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.