ssssssssssssssssssAcetyl Coenzyme A Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Ultraviolet spectrophotometer

Cat No: BC0980

Size:50T/48S

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	Preservation Condition
Extraction I	Liquid 60 mL×1	2-8℃
Extraction II	Liquid 600 μL×1	-20℃
Reagent I	Powder×2	-20℃
Reagent II	Liquid 10 μL×2	2-8℃
Reagent IIIA	Liquid 50 mL×1	2-8℃
Reagent IIIB	Powder×2	-20℃
Reagent IV	Liquid 5 mL×1	2-8℃

Solution Preparation:

1. Reagent I: Add 325 μL Reagent IV before use. It could be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.
2. Reagent II: Take one Reagent II to centrifugal before use. Add 250μL Reagent IV and dissolve well. It could be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.
3. Reagent III: Add 24 mL Reagent IIIA to one Reagent IIIB before use. It could be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.
4. Working solution: Before use, prepare according to the sample size in the ratio of Reagent I: Reagent II: Reagent III=1: 1: 90.

Description:

Acetyl coenzyme a is an important intermediate metabolite in the process of energy metabolism, which is widely found in animals, plants, microbes and cultured cells. Three major nutrients (sugar, fat and protein) converge through acetyl coenzyme a to form a common metabolic pathway - tricarboxylic acid cycle and oxidative phosphorylation. Through this pathway, they are completely oxidized to produce carbon dioxide and water, release energy for ATP synthesis. Acetyl coenzyme a is the precursor for synthesis of bioactive substances such as fatty acids, ketones, cholesterol and their derivatives.

Malate Dehydrogenase (MDH) catalyzes NAD^+ and malate to generate NADH and oxaloacetate. Citrate Synthase (CS) catalyzes oxaloacetate and acetyl coenzyme a to generate Citrate and Coenzyme A. Because of the coupling reaction of MDH and CS, acetyl coenzyme a content is proportional to NADH production rate. Acetyl coenzyme a could be calculated by changes of light absorption at 340nm.

Required but not provided:

Ultraviolet spectrophotometer, balance, constant temperature foster box/water-bath, centrifuge, 1mL quartz cuvette, transferpettor, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure:

I. Sample Preparation.

1. **Tissue:** Suggest that weigh 0.1 g of sample, add 0.99 mL of Extraction I, 0.01 mL of Extraction II and homogenate in ice bath. Centrifuge at 4℃ and 8000g for 10 minutes and discard precipitation, take the supernatant on ice for test.
2. **Cells or bacteria:** Collect 5 million bacteria or cells into a centrifuge tube, add 0.99 mL of Extraction I and 0.01 mL of Extraction II to ultrasonically break bacteria or cells (power 200W, ultrasonic 3s, 10s interval, repeat 30 times). Centrifuge at 4℃and 8000g for 10 minutes and discard precipitation, take the supernatant on ice for test.
3. **Serum (plasma) and other liquid:** Detect directly. Centrifuge before detect if there are precipitation in the liquid.

II. Determination Procedure:

1. Preheat ultraviolet spectrophotometer for 30min, adjust wavelength to 340 nm, set counter to zero with distilled water.
2. Preheat Working solution at 37℃(mammals) or 25℃ (other species) for 10min.
3. Add 200 μL supernatant of samples and 820 μL working solution into 1mL quartz cuvette. Mix thoroughly. Record the initial absorbance A1 at the wavelength of 340 nm for 20 seconds, after 1 min's reaction record absorbance value A2 for 80s. ΔA=A2-A1.

III. Acetyl Coenzyme A Content Calculation

1. Sample weight

   Acetyl Coenzyme A Content (nmol/g weight) $=(\Delta A\div\varepsilon\div d)\times V_R\times10^9\div V_S\times V_E\div W\times F=819.9\times\Delta A\div W\times F$
2. Germ or cells

   Acetyl Coenzyme A Content (nmol/10^4 cell) $=(\Delta A\div\varepsilon\div d)\times V_R\times10^9\div V_S\times V_E\div500\times F=1.640\times\Delta A\times F$
3. Serum (plasma) and other liquid

   Acetyl Coenzyme A Content (nmol/mL) $=(\Delta A\div\varepsilon\div d)\times V_R\times10^9\div V_S\times F=819.9\times\Delta A\times F$

   ε: The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;

   d: The light diameter of cuvette, 1 cm;

   V_R: Total reaction volume, 1.02×10^{-3} L;

   10^9: Unit conversion factor, 1 mol=10^9 nmol;

   Vs: Sample volume, 0.2 mL;

   V_E: Add the volume of Extraction I and Extraction II, 1 mL;

   W: Sample weight, g;

   500: Cells or germ, 5 million;

F: Dilution times.

Experimental example:

1. Take 0.1g rat kidney, add 0.99 mL of Extraction I and 0.01 mL of Extraction II, grind the homogenate with ice bath, centrifuge at 4℃ and 8000g for 10min, and place the supernatant on ice. Then operate according to the determination steps, calculate ΔA = A2-A1 = 0.667-0.745=0.078, and calculate the enzyme activity according to the sample mass:
   Acetyl Coenzyme A Content (nmol/g weight) =819.9×0.078÷0.1=639.5 nmol/g weight.

Recent Product Citations:

[1] Li W, Huang X, Liu H, Lian H, Xu B, Zhang W, Sun X, Wang W, Jia S, Zhong C. Improvement in bacterial cellulose production by co-culturing Bacillus cereus and Komagataeibacter xylinus. Carbohydr Polym. 2023 Aug 1;313:120892. doi: 10.1016/j.carbpol.2023.120892. Epub 2023 Apr 11. PMID: 37182977.

[2] Zhang N, Song L, Xu Y, Pei X, Luisi BF, Liang W. The decrotonylase FoSir5 facilitates mitochondrial metabolic state switching in conidial germination of Fusarium oxysporum. Elife. 2021 Dec 20;10:e75583. doi: 10.7554/eLife.75583. PMID: 34927582; PMCID: PMC8730727.

[3] Hu L, Guo C, Chen J, Jia R, Sun Y, Cao S, Xiang P, Wang Y. Venturicidin A Is a Potential Fungicide for Controlling Fusarium Head Blight by Affecting Deoxynivalenol Biosynthesis, Toxisome Formation, and Mitochondrial Structure. J Agric Food Chem. 2023 Aug 23;71(33):12440-12451. doi: 10.1021/acs.jafc.3c02683. Epub 2023 Aug 10. PMID: 37566096.

[4] Cao W, Zhang L, Wu L, Zhang M, Liu J, Xie Z, Liu H. Identification and genetic characterization of mitochondrial citrate transporters in Aspergillus niger. Front Microbiol. 2022 Sep 13;13:1009491. doi: 10.3389/fmicb.2022.1009491. PMID: 36177470; PMCID: PMC9512666.

[5] Liu J, Zhang S, Li W, Wang G, Xie Z, Cao W, Gao W, Liu H. Engineering a Phosphoketolase Pathway to Supplement Cytosolic Acetyl-CoA in Aspergillus niger Enables a Significant Increase in Citric Acid Production. J Fungi (Basel). 2023 Apr 23;9(5):504. doi: 10.3390/jof9050504. PMID: 37233215; PMCID: PMC10219267.

Related Products:

BC0710/BC0715	α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit
BC2150/BC2155	Citric Acid (CA) Content Assay Kit
BC0950/BC0955	Succinate Dehydrogenase (SDH) Activity Assay Kit
BC0380/BC0385	Pyruvate Dehydrogenase (PDH) Activity Assay Kit
BC2160/BC2165	Isocitrate Dehydrogenase Mitochondrial (ICDHm) Activity Assay Kit