Acetyl Coenzyme A Content Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer/Microplate reader

Cat No: BC0985

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	Preservation Condition
Extraction I	Liquid 110 mL×1	2-8℃
Extraction II	Liquid 0.6mL×2	-20℃
Reagent I	Powder×2	-20℃
Reagent II	Liquid 5 μL×2	2-8℃
Reagent IIIA	Liquid 25 mL×1	2-8℃
Reagent IIIB	Powder×2	-20℃
Reagent IV	Liquid 5 mL×1	2-8℃

Solution Preparation:

1. Reagent I: Add 163 μL Reagent IV to one Reagent I 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 125μL 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 12 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/microplate reader, balance, water-bath/constant temperature foster box, centrifuge, transferpettor, micro quartz cuvette/96 well UV plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol:

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/microplate reader for 30min, adjust wavelength to 340 nm, set spectrophotometer counter to zero with distilled water.
2. Preheat Working solution at 37℃(mammals) or 25℃(other species) for 10min.
3. Add 50 μL supernatant of samples and 205 μL working solution into micro quartz cuvette/96 well UV plate. 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

A. micro quartz cuvette

1. Sample weight

   Acetyl Coenzyme A Content (nmol/g weight)=$(\Delta A \div \varepsilon \div d) \times V_R \times 10^9 \div V_S \times V_E \div W \times F = 819.9 \times \Delta A \div W \times F$
2. Germ or cells

   Acetyl Coenzyme A Content (nmol/10^4 cell)=$(\Delta A \div \varepsilon \div d) \times V_R \times 10^9 \div V_S \times V_E \div 500 \times F = 1.640 \times \Delta A \times F$
3. Serum (plasma) and other liquid

   Acetyl Coenzyme A Content (nmol/mL)=$(\Delta A \div \varepsilon \div d) \times V_R \times 10^9 \div V_S \times F = 819.9 \times \Delta A \times F$

   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, 2.55×10^{-4} L;

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

   Vs: Sample volume, 0.05 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.

B. 96 well UV plate

Modify d-1 cm in the above formula to d-0.6 cm (light path of 96 well UV plate) for calculation.

Experimental example:

1. Take 0.1g rabbit 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 = 0.8083-0.7691=0.0392, and calculate the enzyme activity according to the sample mass:
   Acetyl Coenzyme A Content (nmol/g weight)=819.9×0.0392÷0.1×2=642.8 nmol/g weight.

Recent Product Citations:

[1] Sun J, Lin Z, Liao Z, Wu Z, Li H, Wang H. Small extracellular vesicles derived from human adipose-derived stem cells regulate energetic metabolism through the activation of YAP/TAZ pathway facilitating angiogenesis. Cell Biol Int. 2023 Feb;47(2):451-466. doi: 10.1002/cbin.11938. Epub 2022 Oct 23. PMID: 36279478.

[2] Zeng M, Shao C, Zhou H, He Y, Li W, Zeng J, Zhao X, Yang J, Wan H. Protocatechudehyde improves mitochondrial energy metabolism through the HIF1/PDK1 signaling pathway to mitigate ischemic stroke-elicited internal capsule injury. J Ethnopharmacol. 2021 Sep 15;277:114232. doi: 10.1016/j.jep.2021.114232. Epub 2021 May 24. PMID: 34044078.

[3] Xie D, Sun Y, Lei Y. Effect of glucose levels on carbon flow rate, antioxidant status, and enzyme activity of yeast during fermentation. J Sci Food Agric. 2022 Sep;102(12):5333-5347. doi: 10.1002/jsfa.11887. Epub 2022 Apr 27. PMID: 35318660.

[4] Liang Y, Zhao L, Dai C, Liu G, Zhong Y, Liu H, Mo L, Tan C, Liu X, Chen L. Epileptiform Discharges Reduce Neuronal ATP Production by Inhibiting F0F1-ATP Synthase Activity via A Zinc-2-Glycoprotein-Dependent Mechanism. Mol Neurobiol. 2023 Nov;60(11):6627-6641. doi: 10.1007/s12035-023-03508-3. Epub 2023 Jul 20. PMID: 37468739.

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