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ATP-Citrate Lyase Assay Kit, UV, 48 Tests

Product Number: BCK0923

Shipping and Storage

1. Reagent 1: Store at −20°C and protected from light;
2. Reagent 3: Store at −20°C;
3. Extraction Solution, Reagent 2: Store at 4°C.
4. Validity period: 3 months.

Component

Component	48T
Extraction Solution	60mL× 1 bottle
Reagent 1	Powder, 2 vials
Reagent 2	30mL× 1 bottle
Reagent 3	Powder, 4 vials

1. Reagent 1: Ensure powder settles to the bottom before opening (manual flicking is acceptable). Reconstitute each vial with an additional 1.1mL distilled water. The reconstituted reagent can be aliquoted and stored at −20°C.
2. Reagent 3: Ensure powder settles to the bottom before opening (manual flicking is acceptable). Add 3.9mL distilled water for reconstitution. Aliquot unused reagent and store at −20°C. Avoid repeated freeze-thaw cycles and use within three days.

Description

ATP-citrate lyase (ACL, EC 4.1.3.8) is a key enzyme in carbohydrate metabolism and fatty acid biosynthesis. Its substrates and products are vital intermediates in carbohydrate metabolism and can serve as substrates for fatty acid synthesis. Meanwhile, it plays an important role in plant growth and development as well as improving plant stress resistance. In addition, ACL is also a key enzyme in the tricarboxylic acid cycle.

In the presence of ATP and coenzyme A, ACL catalyzes the cleavage of citrate into acetyl-CoA, oxaloacetate, adenosine diphosphate and phosphate. Malate dehydrogenase further catalyzes the reaction between oxaloacetate and NADH to produce malate and NAD^+. The rate of NADH consumption is measured at 340nm to calculate ACL enzyme activity.

Required Laboratory Equipment

Mortar (homogenizer), ice box (ice-maker), table-top centrifuge, adjustable pipettors, water bath (oven / incubator / metal heat block), 1mL quartz cuvettes, centrifuge tubes, UV-visible spectrophotometer, distilled water (deionized water or ultrapure water is acceptable).

Sample Assay

It is recommended to run a pilot test with 1-3 representative samples of distinct groups/types to get familiar with the workflow. Adjust sample loading concentration according to pilot results to avoid unnecessary consumption of samples and reagents.

1. Sample Extraction

1.1. Tissue Samples

Weigh approximately 0.1 g of tissue (0.5 g can be taken for fruit samples with high water content), add 1mL of extraction solution, and homogenize on ice. Centrifuge at 12000 rpm, 4°C for 10 min, collect the supernatant and keep it on ice for testing.

Note: Extraction may also be performed at a tissue mass (g) to extraction solution volume (mL) ratio of 1:5~10.

1.2. Liquid samples: Test directly. If turbid, centrifuge and collect the supernatant for testing.

1.3. Bacterial / Cell Samples

Collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant. Add 1mL of extraction solution to 5 million bacteria or cells. Sonicate bacteria or cells on ice (power 20% or 200 W, sonicate for 3 s, interval 10 s, repeat 30 times). Centrifuge at 12000 rpm, 4°C for 10 min, collect the supernatant and keep it on ice for testing.

Note: For larger sample input, perform extraction at ratio: cell count ($\times 10^4$ cells) : Extraction Solution (mL) = 500~ 1000 :1. Cell lysis using commercial lysis buffer is also acceptable; follow the lysis-buffer manufacturer's instructions.

2. Protocol

2.1. Warm up the microplate reader for no less than 30 min (waiting for the instrument to complete self-test is also acceptable). Set the wavelength to 340nm and the temperature to 25°C.

2.2. Reagents 2 and 3 taken out from low temperature may be incubated in a 25°C water bath for 10 min.

2.3. Add sequentially into a 1mL quartz cuvette (1 cm light path):

Component	Assay tube
Sample	40μL
Reagent 1	40μL
Reagent2	440μL
Reagent 3	270μL
Mix thoroughly. Immediately read absorbance A1 at 340 nm at room temperature (25°C), then read A2 after 10 min. ΔA = A1 − A2.	

Note: If the ΔA value fluctuates near zero, extend the reaction time from 10 min to 30 min, and substitute the corresponding reaction time T into the calculation formula for recalculation. Alternatively, increase the sample loading volume (e.g., from 40 μL to 80 μL). Substitute the adjusted sample loading volume V_1 and total reaction volume V_2 into the formula for recalculation. Another option is to increase the sampling mass from 0.1g to 0.2g, add 1mL of extraction solution for homogenization and extraction, and substitute the revised sample weight W into the formula for calculation.

If the decreasing trend is unstable, read the absorbance every 10 seconds, select a time period with linear decrease for calculation, and substitute the corresponding A values into the formula for recalculation.

If the initial value A_1 is excessively high and exceeds 2 (e.g., deeply colored plant leaves usually contain high pigments and tend to yield high initial absorbance), appropriately reduce the sample loading volume and substitute the revised loading volume into the formula for recalculation. Alternatively, add a small amount of activated carbon to the test sample, mix and stand for 5 min, then centrifuge at 12000 rpm, 4°C for 10 min. Use the supernatant for detection.

Calculation of Results

1. Calculated based on the fresh weight of the sample:

Enzyme activity definition: One unit of enzyme activity is defined as the consumption of 1nmol NADH per gram of tissue per minute.

ACL (nmol/min/g fresh weight) = $[\Delta A \times V2 \div (\varepsilon \times d) \times 10^9 \div (W \times V1 \div V) \div T = 321.5 \times \Delta A \div W$

2. Calculated based on the protein concentration of the sample:

Enzyme activity definition: One unit of enzyme activity is defined as the consumption of 1 nmol NADH per milligram of tissue protein per minute.

ACL (nmol/min/mg prot) = $[\Delta A \times V2 \div (\varepsilon \times d) \times 10^9] \div (V1 \times Cpr) \div T = 321.5 \times \Delta A \div Cpr$

3. Calculated based on the volume of the liquid sample:

Enzyme activity definition: One unit of enzyme activity is defined as the consumption of 1 nmol NADH per milliliter of liquid sample per minute.

ACL(nmol/min/mL)=$[\Delta A \times V2 \div (\varepsilon \times d) \times 109] \div V1 \div T = 321.5 \times \Delta A$

4. Calculated based on bacterial/cell density:

Enzyme activity definition: One unit of enzyme activity is defined as the consumption of 1 nmol NADH per 10,000 cells per minute.

ACL (nmol/min/10^4 cell) = $[\Delta A \times V2 \div (\varepsilon \times d) \times 10^9] \div (500 \times V1 \div V) \div T = 0.64 \times \Delta A$

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Parameter explanations:

ε — Molar extinction coefficient of NADH, 6.22×10^{9} L/mol/cm;

V — Volume of extraction solution added, 1mL;

V1 — Volume of sample added, 0.04mL;

V2 — Total volume of reaction system, 8×10^{-4} L;

T — Reaction time, 10 min;

d — Light path, 0.5 cm;

500 — Total number of bacteria or cells, 10^{4};

W — Sample weight, g;

Cpr — Protein concentration of sample, mg/mL; The BCA protein assay kit from our company is recommended.