MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

ATP-Citrate Lyase Assay Kit, Microplate, 96 Tests

Product Number: BCK0924

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	96T
Extraction Solution	120mL× 1 bottle
Reagent 1	1 EP tube
Reagent 2	15mL× 1 bottle
Reagent 3	Powder, 4 vials

1. Reagent 1: Centrifuge at 8000 g, 4°C for 2 min before use to collect reagent at the bottom of the tube (manual flicking is acceptable). Add 1.1mL distilled water for reconstitution. The reconstituted reagent can be aliquoted and stored at −20°C.
2. Reagent 3: For each vial: Ensure powder settles to the bottom before opening (manual flicking is acceptable). Reconstitute each vial with 1.9mL distilled water. 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 key 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 enhancing plant stress resistance. In addition, ACL is also a key enzyme of 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 conversion of oxaloacetate and NADH to malate and NAD^+. The rate of NADH decrease is measured at 340 nm to determine the activity of ACL.

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 1 mL 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 340 nm 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. Pipette reagents sequentially into wells of a 96-well microplate:

Component	Assay tube
Sample	10μL
Reagent 1	10μL
Reagent2	110μL
Reagent 3	70μL
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. Substitute the corresponding reaction time T into the calculation formula for recalculation. Alternatively, increase the sample loading volume (e.g., increase from 10μL to 20μL while reducing Reagent III accordingly to maintain a total volume of 200μL), and substitute the modified loading volume V_1 into the formula for recalculation. Another option is to increase the sampling mass from 0.1 g to 0.2 g, add 1 mL of extraction solution for homogenization and extraction, and substitute the adjusted 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 1 nmol NADH per gram of tissue per minute.

ACL (nmol/min/g fresh weight) = $[\Delta A \times V2 \div (\varepsilon \times d) \times 10^9] \div (W \times V1 \div V) \div T = 643.1 \times \Delta A \div W$

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) = $[\Delta A \times V2 \div (\varepsilon \times d) \times 10^9] \div (V1 \times Cpr) \div T = 643.1 \times \Delta A \div Cpr$

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)=$[\Delta A \times V2 \div (\varepsilon \times d) \times 109] \div V1 \div T = 643.1 \times \Delta A$

4. Calculated based on bacterial/cell density:

Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

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) = [ΔA × V2 ÷ (ε × d) × 10^9] ÷ (500 × V1 ÷ V) ÷ T = 1.29 × ΔA

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, 0.01mL;

V2 — Total volume of reaction system, 2×10^{-4} L;

T — Reaction time, 10 min;

d — Light path of 96-well plate, 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.