Acetyl-CoA Carboxylase Assay Kit

Catalog No.: abx298890

Size: 96 tests

Detection Range: 6.78 U/L – 138 U/L

Sensitivity: 6.78 U/L

Storage: Store all components at -20°C. Store the Substrate A, Substrate B, Chromogenic Reagent A, and Chromogenic Reagent B in the dark.

Application: For detection and quantification of Acetyl-CoA Carboxylase activity in plant and animal tissue homogenates.

Introduction

Acetyl-CoA Carboxylase is the first enzyme in the fatty acid synthesis pathway, and plays a key role in the metabolism of animals, plants, and prokaryotes. Acetyl-CoA Carboxylase catalyzes the conversion of acetyl-CoA to malonyl-CoA, which is then used in the production of fatty acid chains. Fatty acids themselves play a central role in numerous diseases, including cancers and long-term health conditions like obesity. As such Acetyl-CoA Carboxylase activity, which is a key regulator of fatty acid production, is of intense interest to researchers studying these complex conditions.

Abbexa's Acetyl-CoA Carboxylase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Acetyl-CoA Carboxylase activity. The action of Acetyl-CoA Carboxylase on certain substrates produces inorganic phosphorous, which then reacts with the chromogenic reagents provided in this kit to a produce a compound with an absorbance maximum at 660 nm. The intensity of the color is proportional to the Acetyl-CoA Carboxylase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 30 ml
3. Substrate A: 2 vials
4. Substrate B: 3.5 ml
5. Acid Solution: 1.8 ml
6. Negative Control: 3.5 ml
7. Chromogenic Reagent A: 2 vials
8. Chromogenic Reagent B: 2 vials
9. Standard (500 µmol/L): 6 ml
10. Plate sealer: 2

Materials required but not provided

1. Microplate reader (660 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Water bath (up to 95°C)
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh out a section of tissue, and add into normal saline (0.9% NaCl) in a ratio of 1:9 weight to volume (i.e. for 1 g of tissue, add 9 ml normal saline). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Centrifuge at 10,000 × g for 10 minutes at 4°C, then take carefully take the supernatant for detection, without disturbing any of the sediment. Assay immediately.

  Note: To calculate enzyme activity in tissue homogenates using the formula provided in **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% *Epipremnum aurem* tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Potato tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate A Working Solution:** Add 5 ml double distilled water to a vial of Substrate A. Mix gently to ensure the powder has fully dissolved. The prepared solution can be stored for up to 7 days at -20°C in the dark.
- **Chromogenic Reagent A Working Solution:** Add 1 ml double distilled water to the vial of Chromogenic Reagent A. Mix gently to ensure the powder has fully dissolved. The prepared solution can be stored for up to 7 days at 4°C in the dark.
- **Chromogenic Reagent B Working Solution:** Add 1 ml double distilled water to the vial of Chromogenic Reagent B. Mix gently to ensure the powder has fully dissolved. The prepared solution can be stored for up to 7 days at 4°C in the dark.
- **Assay Reagent:** In a clean glass container, prepare the Assay Reagent by mixing Double distilled water, Chromogenic Reagent A Working Solution, Chromogenic Reagent B Working Solution, and Acid Solution in a ratio of 2:1:1:1. The Assay Reagent should be a pale yellow color. Discard the solution if it turns green or blue. Keep the prepared solution in the dark.
- **Standards:** Label 7 tubes with 500 µmol/L, 450 µmol/L, 400 µmol/L, 300 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L. Add 200 µl, 180 µl, 160 µl, 120 µl, 80 µl, 60 µl, and 40 µl of Standard (500 µmol/L) to the 500 µmol/L, 450 µmol/L, 400 µmol/L, 300 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L tubes respectively, followed by 0 µl, 20 µl, 40 µl, 80 µl, 120 µl, 140 µl, and 160 µl of double distilled water, to prepare Standard Dilutions with concentrations 500 µmol/L, 450 µmol/L, 400 µmol/L, 300 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**500**	**450**	**400**	**300**	**200**	**150**	**100**
500 µmol/L Standard (µl)	200	180	160	120	80	60	40
Double distilled water (µl)	0	20	40	80	120	140	160

For the blank, or 0 µmol/L standard, use pure Double distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- If there is any precipitate present in the Chromogenic Reagent B Working Solution, heat the vial to > 90°C and stir gently to dissolve the suspension. Allow the vial to cool back to room temperature before use.
- The Assay Reagent contains phosphorous, and is highly sensitive to impurities found on the surfaces of glass containers. The container for this solution should be as clean as possible. This can be achieved by scrubbing the glass thoroughly, and rinsing it with pure double distilled water at least 10 times.

B. Assay Procedure

1. Mark microcentrifuge tubes for each sample and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 200 µl of Buffer Solution to all tubes.
3. Add 50 µl of Substrate A Working Solution to all tubes.
4. Add 40 µl of Negative Control to the control tubes.
5. Add 40 µl of Substrate B to the sample tubes.
6. Add 40 µl of sample to the samples tubes and the control tubes.
7. Mix fully, then incubate all the tubes at 37°C for 30 minutes.
8. Heat the tubes in a 95°C water bath for 5 minutes.
9. Centrifuge at 8000 × g for 5 minutes.
10. Mark positions on the microplate for each standard, sample, and control. *It is strongly recommended to prepare the standards in duplicate.*
11. Carefully take 80 µl of supernatant from each tube, and transfer to the corresponding sample and control wells on the 96-well microplate.
12. Add 80 µl of each standard dilution to the corresponding standard wells on the 96-well microplate.
13. Add 50 µl of Assay Reagent to all wells.
14. Shake the plate with a microplate shaker to ensure the well contents are mixed fully, and then cover with a plate sealer and incubate at 37°C for 10 minutes.
15. Measure the OD of each well with a microplate reader at 660 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Acetyl-CoA Carboxylase in each sample well can be derived with the following formula:

Tissue samples

One unit of Acetyl-CoA Carboxylase activity is defined as the amount required for 1 g of tissue protein to produce 1 µmol inorganic phosphorous per minute at 37°C.

$$\text{Acetyl-CoA Carboxylase (U/g protein)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Reaction}}}{\mathrm{a} \times \mathrm{t} \times \mathrm{V_{Sample}} \times \mathrm{C_{Protein}}}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
V_{Sample}	Volume of sample in enzymatic reaction (0.04 ml)
$V_{Reaction}$	Volume of all reagents in enzymatic reaction (0.33 ml)
$C_{Protein}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (30 mins)
F	The dilution factor of sample