Acetyl-CoA Assay Kit

Catalog No.: abx294402

Size: 96 tests

Detection Range: 150 nmol/ml – 500 nmol/ml

Sensitivity: 150 nmol/ml

Storage: Store all components at -20°C. The Enzyme Reagent A, Enzyme Reagent B, Substrate, and Standard (500 nmol/ml) should be stored in the dark.

Application: For detection of Acetyl-CoA concentrations in tissue homogenates.

Introduction

Acetyl-CoA is a vital cofactor in posttranslational modifications, such as acetylation of histone and nonhistone proteins. Acetyl-CoA can be produced through oxidative decarboxylation of pyruvate in glycolysis, or by oxidative degradation of amino acids. Acetyl-CoA will subsequently enter the citric acid cycle and be used to produce ATP.

Abbexa's Acetyl-CoA Assay Kit is a quick, convenient, and sensitive method for the detection of Acetyl-CoA concentration. The reaction product has an absorbance maxima at 340 nm. The intensity of the color is proportional to the Acetyl-CoA concentration which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 60 ml
3. Buffer Solution: 40 ml
4. Enzyme Reagent A: 2 vials
5. Enzyme Reagent B: 2 vials
6. Substrate: 2 vials
7. Standard (500 nmol/ml): 0.32 ml
8. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (340 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use.

- **Enzyme Reagent A Working Solution**: Dissolve 1 vial of Enzyme Reagent A with 500 µl of Buffer Solution. Mix well until dissolved. Aliquot and store at -20°C in the dark for up to 3 days. Avoid repeated freeze/thaw cycles.
- **Enzyme Reagent B Working Solution**: Dissolve 1 vial of Enzyme Reagent B with 500 µl of double-distilled water. Mix well until dissolved. Store at 2-8°C in the dark for up to 3 days.
- **Substrate Working Solution**: Dissolve 1 vial of Substrate with 500 µl of Buffer Solution. Mix well until dissolved. Store at 2-8°C in the dark for up to 3 days.
- **Reaction Working Solution**: Mix 400 µl of Buffer Solution, 14 µl of Enzyme Reagent A Working Solution, 10 µl of Enzyme Reagent B Working Solution, and 12 µl of Substrate Working Solution to obtain 436 µl of Reaction Working Solution. Mix well. Prepare sufficient solution for the number of wells being used. Prepare just before use and avoid exposure to light. Bring the solution to room temperature in the dark for 10 minutes prior to use in the assay.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

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

- **Tissue Homogenates:** Weigh 20 mg of tissue and wash with pre-chilled PBS (0.01 M, pH 7.4). For each 1 mg of tissue, add 9 µl of Extraction Solution. Homogenize by hand using a mechanical homogenizer, or by ultrasonication at 4°C. Centrifuge the homogenate at 10,000 × g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should 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.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Lung Tissue Homogenate	1
10% Rat Liver Tissue Homogenate	1
10% Rat Spleen Tissue Homogenate	1
10% Rat Kidney Tissue Homogenate	1
10% Mouse Lung Tissue Homogenate	1
10% Mouse Kidney 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 tissue homogenates.

B. Assay Procedure

1. Bring the Reaction Working Solution to room temperature in the dark for 10 minutes prior to use.
2. Set standard, sample and blank wells on the microplate and record their positions. We recommend setting up each standard, sample and blank in duplicate. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
3. Add 30 µl of Standard Solution to the standard wells.
4. Add 30 µl of Extraction Solution to the blank wells.
5. Add 30 µl of the sample to the sample wells.
6. Add 230 µl of Reaction Working Solution to each well and mix well.
7. Measure the OD value of each well at 0 min and 1 min at 340 nm with the microplate reader. (A_1 = OD value at 0 min, A_2 = OD value at 1 min. $\Delta A = A_2 - A_1$).

C. Calculation of Results

Tissues samples:

$$\text{Acetyl CoA content (nmol/g wet weight)} = \frac{(\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}})}{(\Delta A_{\text{Standard}} - \Delta A_{\text{Blank}})} \times \frac{C \times V}{m} \times f$$

where:

ΔA_{Sample}	Change in OD value of the sample wells
$\Delta A_{\text{Standard}}$	Change in OD value of the standard wells
ΔA_{Blank}	Change in OD value of the blank wells
C	Concentration of standard (500 nmol/ml)
m	Weight of wet tissue (g)
V	Volume of homogenate (ml)
f	Dilution factor of sample prior to carrying out the assay