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Product Information

Acetyl-Coenzyme A Assay Kit

Catalog Number **MAK039**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Acetyl-CoA is an essential cofactor and carrier of acyl groups in enzymatic acetyl transfer reactions. It is formed either by the oxidative decarboxylation of pyruvate in mitochondria, by the oxidation of long-chain fatty acids, or by the oxidative degradation of certain amino acids. Acetyl-CoA is the starting compound for the citric acid cycle (Kreb′s cycle). It is also a key precursor in lipid biosynthesis and the source of all fatty acid carbons. Acetyl-CoA positively regulates the activity of pyruvate carboxylase. It is a precursor of the neurotransmitter acetylcholine. Histone acetylases (HAT) use Acetyl-CoA as the donor for the acetyl group used in the post-translational acetylation reactions of histone and non-histone proteins.

This kit is a highly sensitive assay for determining Acetyl-CoA level in a variety of biological samples. Acetyl-CoA concentration is determined by a coupled enzyme assay, which results in a fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the Acetyl-CoA present. This assay can be set up to determine Acetyl-CoA in either the nmole or pmole range.

Components

The kit is sufficient for 100 assays in 96 well plates.

Acetyl-CoA Assay Buffer Catalog Number MAK039A	25 mL
Fluorescent Probe, in DMSO Catalog Number MAK039B	0.2 mL
Conversion Enzyme Catalog Number MAK039C	0.1 mL
Acetyl-CoA Enzyme Mix Catalog Number MAK039D	0.5 mL
Acetyl-CoA Substrate Mix Catalog Number MAK039E	1 vl
Coenzyme A Quencher Catalog Number MAK039F	1 mL
Quench Remover Catalog Number MAK039G	1 vl
Acetyl-CoA Standard, 1 μmole Catalog Number MAK039H	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader
- 1.0 M Perchloric Acid (PCA)
- Potassium Bicarbonate (Catalog Number 60339 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Acetyl-CoA Assay Buffer – Allow buffer to come to room temperature before use.

Fluorescent Probe – Warm to room temperature prior to use to melt DMSO. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C.

Acetyl-CoA Substrate Mix – Reconstitute in 220 μL of Acetyl-CoA Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Quench Remover – Reconstitute in 220 µL of water. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use.

Acetyl-CoA Standard – Reconstitute in 100 µL of water to generate 10 mM standard solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Acetyl CoA Standards for Fluorometric Detection
Assay Range (0–1 nmole): Dilute 10 µL of the 10 mM Acetyl CoA standard solution with 990 µL of water to prepare a 0.1 mM standard solution. Dilute 100 µL of the 0.1 mM solution into 400 µL of water to prepare a 0.02 mM standard solution. Add 0, 10, 20, 30, 40, and 50 µL of the 0.02 mM Acetyl CoA standard solution into appropriate wells of a 96 well plate, generating 0 (blank), 200, 400, 600, 800, and 1000 pmole/well standards. Add Acetyl-CoA Assay Buffer to each well to bring the volume to 50 µL.

Assay Range (0–100 pmole): Dilute 10 µL of the 10 mM Acetyl CoA standard solution with 990 µL of water to prepare a 0.1 mM standard solution. Dilute 10 µL of the 0.1 mM standard solution with 490 µL of water to make a 2 µM standard solution. Add 0, 10, 20, 30, 40, and 50 µL of the diluted Acetyl CoA standard into appropriate wells of a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add Acetyl-CoA Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue samples (20–1,000 mg) should be frozen rapidly (liquid N_2 or methanol/dry ice) and pulverized.

Deproteinize sample by PCA precipitation. Add 2 µL of 1 M perchloric acid/mg of sample, keeping sample cold. Homogenize or sonicate thoroughly. Centrifuge the samples at 10,000 × *g* for 10 minutes to remove insoluble material. Neutralize the supernatant with 3 M potassium bicarbonate solution, adding in aliquots of 1 µL/10 µL of supernatant while vortexing until bubble evolution ceases (2–5 aliquots). Cool on ice for 5 minutes. Verify pH is in the range of 6–8, using 1 µL of sample. Spin 2 minutes to pellet potassium bicarbonate.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Add 10 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Acetyl-CoA Assay Buffer.

Include a blank sample for each sample by omitting the Conversion Enzyme in the Reaction Mix.

To correct for background created by free Coenzyme A and succinyl-CoA, add 10 µL of Acetyl-CoA Quencher to each sample, standard, and sample blank well. Incubate at room temperature for 5 minutes. Add 2 µL of Quench Remover, mix well, and incubate an additional 5 minutes.

Assay Reaction
1. Set up the appropriate Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Assay Range (0–1 nmole)

Reagent	Samples and Standards	Blank Sample
Acetyl-CoA Assay Buffer	40 µL	41 µL
Acetyl-CoA Substrate Mix	2 µL	2 µL
Conversion Enzyme	1 µL	–
Acetyl-CoA Enzyme Mix	5 µL	5 µL
Fluorescent Probe	2 µL	2 µL

Assay Range (0–100 pmole)

Reagent	Samples and Standards	Blank Sample
Acetyl-CoA Assay Buffer	41.8 µL	42.8 µL
Acetyl-CoA Substrate Mix	2 µL	2 µL
Conversion Enzyme	1 µL	–
Acetyl-CoA Enzyme Mix	5 µL	5 µL
Fluorescent Probe	0.2 µL	0.2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 10 minutes at 37 °C. Protect the plate from light during the incubation.

3. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Acetyl-CoA standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Acetyl-CoA standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected fluorescence measurement. Using the corrected fluorescence measurement, the amount of Acetyl-CoA present in the sample may be determined from the standard curve.

Concentration of Acetyl-CoA

$$A_y/S_v = C$$

A_y = Amount of Acetyl-CoA in unknown sample (pmole) from standard curve

S_v = Sample volume (μL) added into the wells.

C = Concentration of Acetyl-CoA in sample

Acetyl Coenzyme A molecular weight: 809.6 g/mole.

Sample Calculation

Amount of Acetyl-CoA (A_y) = 25.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Acetyl-CoA in sample

$$25.84 \text{ nmole}/50\ \mu\text{L} = 0.5168 \text{ nmole}/\mu\text{L}$$

$$0.5168 \text{ nmole}/\mu\text{L} \times 809.6 \text{ ng/nmole} = 418.10 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer to and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use PCA precipitation to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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