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Product Information

Coenzyme A Assay Kit

Catalog Number **MAK034**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Coenzyme A (CoA) is an essential metabolic cofactor synthesized from cysteine, pantothenate, and ATP. CoA plays important roles in many metabolic pathways, including the tricarboxylic acid cycle, and the synthesis and oxidation of fatty acids. One of the main functions of CoA is the carrying and transfer of acyl groups. Acylated deriviates, for example acetyl-CoA, are critical intermediates in many metabolic reactions. CoA levels can be altered during starvation, and in conditions such as cancer, diabetes, and alcoholism.

The Coenzyme A Assay kit is an easy and convenient assay to measure the CoA level in variety biological samples. CoA concentration is determined by an enzymatic assay, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product, proportional to the CoA present. Typical detection range for this kit is 0.2–10 nmoles of CoA.

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

Coenzyme A Assay Buffer Catalog Number MAK034A	25 mL
Fluorescent Peroxidase Substrate Catalog Number MAK034B	0.2 mL
Conversion Enzyme Mix Catalog Number MAK034C	1 vl
Coenzyme A Substrate Mix Catalog Number MAK034D	1 mL
Acyl Coenzyme A Developer Catalog Number MAK034E	1 vl
Coenzyme A Standard, 10 µmole Catalog Number MAK034F	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 and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

Coenzyme A Assay Buffer – Allow buffer to come to room temperature before use.

Fluorescent Peroxidase Substrate – Warm to room temperature before use. Mix well by pipetting. Store at –20 °C, protected from light and moisture.

For the fluorescence assay, dilute an aliquot of the Fluorescent Peroxidase Substrate 4-fold with Coenzyme A Assay Buffer just prior to use. This will reduce the background of the fluorescence assay.

Conversion Enzyme Mix and Acyl Coenzyme A Developer – Reconstitute each in 220 µL of Coenzyme A Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Coenzyme A Standard - Reconstitute in 100 µL of water to generate 100 mM solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

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

Procedure
Coenzyme A Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Coenzyme A Standard with 990 µL of water to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Coenzyme A standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Coenzyme A Assay Buffer to each well to bring the volume to 40 µL.

Coenzyme A Standards for Fluorometric Detection
Prepare a 1 mM standard solution as for the colorimetric assay. Dilute 10 µL of the 1 mM Coenzyme A standard solution with 90 µL of water to make a 0.1 mM Coenzyme A standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM Coenzyme A standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Coenzyme A Assay Buffer to each well to bring the volume to 40 µL.

Sample Preparation
Tissue samples (20–40 mg) should be rapidly homogenized with 100 µL of ice cold PBS or other buffer (pH 6.5–8). Because enzymes in the sample may interfere with the sample reading, it is recommended to deproteinize the samples using a 10 kDa cut-off spin filter. Add between 1–40 µL deproteinized samples into duplicate wells of a 96-well plate and bring to a final volume of 40 µL with Coenzyme A Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Add 10 µL of the Coenzyme A Substrate Mix and 2 µL of Conversion Enzyme Mix to each standard and sample well. Mix well.
   Note: Long chain acyl-CoAs in the sample can generate background in the assay. If significant amounts of acyl-CoAs are in the sample it is advised to do an Acyl-CoA background control. Prepare a blank well for each sample by omitting the Conversion Enzyme from the reaction. The Acyl-CoA blank background should be subtracted from CoA readings.

2. Incubate at 37 °C for 30 minutes.

3. Set up the Master Reaction Mix according to the scheme in Table 1 using diluted Fluorescent Peroxidase Substrate if measuring fluorescence. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Master Reaction Mix
Coenzyme A Assay Buffer	46 µL
Acyl-CoA Developer	2 µL
Fluorescent Peroxidase Substrate	2 µL

4. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

5. For colorometric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) Coenzyme A Standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Coenzyme A standards to plot a standard curve. The amount of Coenzyme A present in the samples may be determined from the standard curve.

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

Concentration of Coenzyme A

$$S_a/S_v = C$$

S_a = Amount of Coenzyme A in unknown sample (nmole) from standard curve

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

C = Concentration of Coenzyme A in sample

Coenzyme A molecular weight: 767.5 g/mole

Sample Calculation

Amount of Coenzyme A (S_a) = 2 nmole

Sample volume (S_v) = 40 μL,

Concentration of Coenzyme A in sample

$$2 \text{ nmole}/40 \ \mu L = 0.05 \text{ nmole}/\mu L$$

$$0.05 \text{ nmole}/\mu L \times 767.5 \text{ ng/nmole} = 38.4 \text{ ng}/\mu l.$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Assay Buffer Ice Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer 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. For colorimetric assays, use clear plates
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 a 10 kDa MWCO spin filter 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 needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
		Deproteinize samples
	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	Always check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always prepare fresh Master Reaction Mix before 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 Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to the dilutions 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
		Deproteinize samples
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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