

Coenzyme A Assay Kit

(Catalog #K367-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Coenzyme A (CoA) is composed of units derived from cysteine, pantothenic acid, and ATP. It plays important roles in the synthesis and oxidation of fatty acids, pyruvate oxidation in the citric acid cycle and many other biological processes. One of the main functions of CoA is the carrying and transfer of acyl groups. One of the most important acyl groups transferred is the acetate group, in which case the molecule is called acetyl-CoA. The acetyl group eventually finds itself incorporated into a variety of molecules such as cholesterol, acetylcholine, melatonin, heme and the TCA cycle intermediates. BioVision has developed an easy, convenient assay to measure the CoA level in variety biological samples. In the assay, CoA is specifically utilized to generate products which react with OxiRed Probe to generate color ($\lambda = 570 \text{ nm}$) and fluorescence ($Ex=535/Em=587 \text{ nm}$). The assay can detect 0.1 to 10 nmol of CoA (2.5-250 μM concentration range) in a variety of samples.

II. Kit Contents:

Components	K367-100	Cap Code	Part Number
CoA Assay Buffer	25 ml	WM	K367-100-1
OxiRed Probe	lyophilized	Red	K367-100-2
DMSO (anhydrous)	0.4 ml	Brown	K367-100-3
Conversion Enzyme Mix	lyophilized	Blue	K367-100-4
CoA Substrate	1 ml	Purple	K367-100-5
Acyl CoA Developer	lyophilized	Green	K367-100-6
CoA Standard (10 μmol)	lyophilized	Yellow	K367-100-7

III. Storage and Handling:

Store kit at -20°C , protect from light. Warm CoA Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions:

OxiRed Probe: Dissolve with 220 μl of DMSO (provided, need to warm up $>18^\circ\text{C}$ to become liquid) before use. Mix well, store at -20°C , protect from light and moisture.

Conversion Enzyme Mix, Acyl CoA Developer: Dissolve with 220 μl CoA Assay Buffer. Pipette up and down to completely dissolve. Store at -20°C . Use within two months.

CoA Standard: Dissolve in 100 μl dH₂O to generate 100 mM (100 nmol/ μl) CoA Standard solution. Keep cold while in use. Store at -20°C .

V. CoA Assay Protocol:

1. CoA Standard Curve Preparations:

Colorimetric assay: Dilute the CoA Standard to 1 nmol/ μl by adding 10 μl of the Standard to 990 μl of dH₂O, mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of standards wells on a 96 well plate. Adjust volume to 40 μl /well with CoA Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the CoA Standard.

Fluorometric assay: Dilute the CoA Standard to 1 nmol/ μl as for the colorimetric assay. Then dilute another 10-fold to 0.1 nmol/ μl by taking 10 μl into 90 μl of dH₂O. Mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of standards wells on a 96 well plate. Adjust volume to 40 μl /well with CoA Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well CoA standard.

2. Sample Preparation:

Tissue samples (100 mg) should be rapidly homogenized with 100 μl ice cold PBS or other buffer (pH 6.5-8). Enzymes in samples may interfere with the assay. We suggest deproteinizing your sample using a perchloric acid/KOH protocol (BioVision, Cat. #K808-200) or 10 kd molecular weight cut off spin columns (BioVision, Cat.# 1997-25). Add 1-40 μl sample into 96-well plate, bring volume to 40 μl with Assay Buffer. We suggest testing several doses of your samples to ensure the readings are within the standard curve range.

3. CoA Conversion:

Add 10 μl of Substrate, 2 μl of Conversion Enzyme Mix[†] to each standard and sample. Mix well.

[†]Long chain acyl-CoA's in the sample can generate background in the assay. If significant amount of acyl-CoA is in your sample, do a background control; omit Conversion Enzyme from the reaction. The acyl-CoA background should be subtracted from CoA readings.

4. Incubate

for 30 minutes at 37°C .

5. Develop:

Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 μl Reaction Mix containing:

CoA Assay Buffer	46 μl
Acyl-CoA Developer	2 μl **
OxiRed Probe	2 μl ***

Add 50 μl of the Reaction Mix to each well containing the CoA Standard and test samples.

**The Acyl-CoA developer recognizes C8 or longer fatty acid chain to generate signal.

***Use 0.5 μl OxiProbe in the fluorometric Assay to decrease fluorescence background and thus increase detection sensitivity, significantly.

6. Incubate

for 30 minutes at 37°C , protect from light.

7. Measure OD

at 570 nm for the colorimetric assay, or Ex/Em=535/589 for the fluorometric assay.

8. Calculation:

Correct background by subtracting the value of the 0 CoA control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot the standard curve. Then apply the sample readings to the standard curve to get CoA amount in the sample wells.

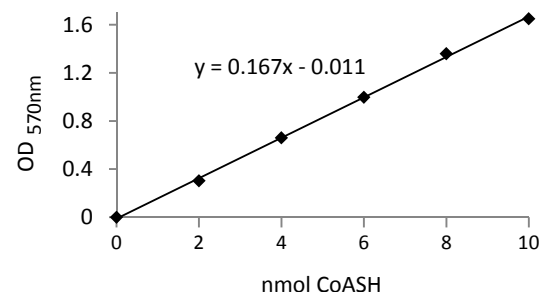
The CoA concentrations in the test samples:

$$C = Ay/Sv \text{ (nmol}/\mu\text{l}; \text{ or } \mu\text{mol}/\text{ml}; \text{ or mM)}$$

Where: Ay is the amount of CoA (nmol) in your sample from the standard curve.

Sv is the sample volume (μl) added to the sample well.

CoA molecular weight: 767.5.



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