BioVision

Coenzyme A (Co-A) Col

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For research use only

(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, free CoA is specifically utilized to generate products which react with OxiRed Probe to generate color (λ = 570 nm) and fluorescence (Ex=535/Em=587 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 (in DMSO)	0.2 ml	Red	K367-100-2A
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: Ready to use as supplied. Please warm up >18 °C to melt frozen DMSO 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 dH_2O 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 dH_2O, 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 dH_2O. 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:** Cells (~1-2 X10^6) or tissue samples (20-40 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 samples 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 your samples contain a significant amount of acyl-CoA, 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 OxiRed Probe in the fluorometric Assay to decrease fluorescence background and increase detection sensitivity.

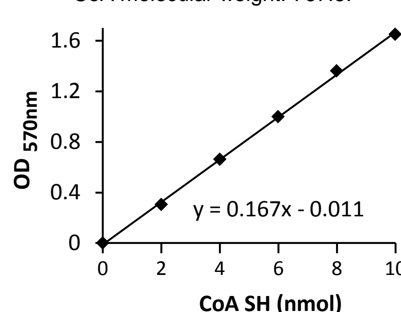
6. Incubate for 30 minutes at 37 °C, protect from light. Measure OD at 570 nm for the colorimetric assay, or Ex/Em=535/589 for the fluorometric assay.

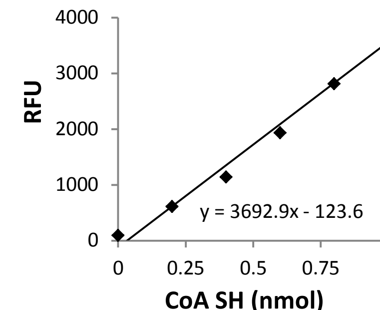
7. **Calculation:** Correct background by subtracting the value of the 0 CoA control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot the standard curve. Then apply the sample readings to the standard curve to get CoA amount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

The CoA concentrations in the test samples:

C = Ay/Sv (nmol/µl; or µmol/ml; 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.





RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
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Glutathione Assay Kit
NAD/NADH and NCOA/NCOAH Assay Kit
TAC Total Antioxidant Capacity
Uric Acid Assay Kit
Ethanol Assay Kit
Cholesterol Assay Kit
Cell Proliferation & Senescence Kits
Cholesterol, LDL/HDL Assay Kits
Ethanol and Uric Acid Assay Kit
Lactate Assay Kits
Mono or Polysaccharide Assay Kits
Fatty Acid Assay Kit
Glycerol Assay Kit
Triglyceride Assay Kit

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		