EnzyChrom™ Coenzyme A Assay Kit (ECOA-100)

Quantitative Colorimetric/Fluorimetric Coenzyme A Determination

DESCRIPTION

Coenzyme A (CoA) is involved in many important biological activities including the synthesis and oxidation of fatty acids, pyruvate oxidation in the citric acid cycle and many others. One of CoA's most crucial roles is the carrying and transferring of acyl groups. BioAssay Systems' method provides a simple, two-step and high-throughput assay for measuring CoA. In this assay, the first step enzymatically converts CoA to acyl-CoA and the second step oxidizes the acyl-CoA producing an enoyl-CoA and H_2O_2. The resulting H_2O_2 reacts with a specific dye to form a pink colored product. The optical density at 570nm or fluorescence intensity (530/585 nm) is directly proportional to the CoA concentration in the sample.

KEY FEATURES

Sensitive. Use 10 μL samples. Linear detection range: colorimetric assay 5 - 1000 μM, fluorimetric assay 3 - 100 μM CoA.

Convenient. Room temperature "mix-and-read" procedure can be readily automated for high-throughput assay of thousands of samples per day.

APPLICATIONS

Assays: CoA in a variety of biological samples.

KIT CONTENTS

Assay Buffer:	20 mL	**Dye Reagent:**	120 μL
Enzyme A:	Dried	**Enzyme B:**	120 μL
Substrate:	600 μL	**Standard**:	50 μL
ATP:	120 μL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation:

Reconstitute Enzyme A by adding 120 μL dH_2O to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down and incubate at RT for 15 min. Store reconstituted Enzyme A at -20°C and use within 2 months.

Colorimetric Assay:

Liquid samples such as serum and plasma can be assayed directly. Milk and solid samples can be homogenized in 5% isopropanol and 5% Triton X-100 in water, followed by filtration through a 0.45μm PTFE syringe filter (e.g. VWR Cat# 28145-493).

Note: SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol, > 5 μM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay. *Important: the thawed Standard solution should be clear and colorless. If the Substrate is turbid, bring it to 37°C and gently swirl the tube (do not vortex) until the solution is clear.*
2. *Standards*: Prepare a 1000 μM stock of standard by diluting 5 μL of the 100 mM Standard with 495 μL Assay Buffer. Dilute the 1000 μM standard in Assay Buffer as follows:

No	1000 μM STD + Buffer	Vol (μL)	Coenzyme A (μM)
1	100 μL + 0 μL	100	1000
2	60 μL + 40 μL	100	600
3	30 μL + 70 μL	100	300
4	0 μL +100 μL	100	0

Transfer 10 μL diluted standards into separate wells of a clear flat-bottom 96-well plate.

Samples: transfer 10 μL of each sample into separate wells of the plate.

3. *ACS reaction*. Prepare enough ACS Working Reagent by mixing, for each well, 40 μL Assay Buffer, 1 μL Enzyme A, 5 μL Substrate, 1 μL ATP. Add 40 μL ACS WR to each well, tap to mix and incubate at room temperature (RT) for 30 min.
4. *ACOD reaction*. Prepare enough ACOD Working Reagent by mixing, for each well, 55 μL Assay Buffer, 1 μL Enzyme B and 1 μL Dye Reagent. Add 50 μL ACOD Working Reagent to each well. Tap plate to mix. Incubate 30 min at room temperature protected from light.
4. Read optical density at 570nm (550-585nm).

Fluorimetric Assay:

The fluorimetric assay procedure is similar to the colorimetric procedure except that (1) 0, 30, 60 and 100 μM Standards and (2) a black 96-well plate are used. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm.

CALCULATION

Subtract Blank value (Standard #4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the fatty acid concentration of Sample,

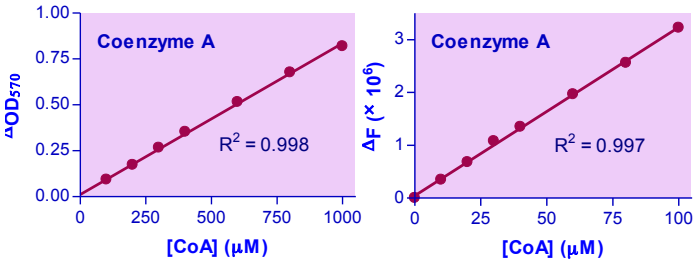
$$[\text{CoA}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope}} \times n \quad (\mu M)$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Blank, respectively. ***n*** is the sample dilution factor.

Note: if the calculated CoA concentration of a sample is higher than 1000 μM in the Colorimetric Assay or 100 μM in the Fluorimetric Assay, dilute sample in Assay Buffer and repeat the assay. Multiply result by the dilution factor, ***n***.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-760), optical density plate reader; black flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-676), fluorescence plate reader. For milk and solid samples, 0.45μm PTFE syringe filter and 5% isopropanol, 5% Triton X-100 solution.



96-well colorimetric assay ***96-well fluorimetric assay***

LITERATURE

1. Veloso D, Veech RL. (1975). Enzymatic determination of long-chain fatty acyl-CoA. Methods Enzymol. 35:273-278.
2. Okabe H, et al. (1980). Enzymic determination of free fatty acids in serum. Clin Chem. 26:1540-1543.
3. Matsubara C, et al. (1983). A spectrophotometric method for the determination of free fatty acid in serum using acyl-coenzyme A synthetase and acyl-coenzyme A oxidase. Anal Biochem. 130:128-133.