

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. Coenzyme A Assay Kit 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 H₂O₂. The resulting H₂O₂ reacts with a specific dye to form a pink colored product. The optical density at 570 nm 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: 120 µL
- ✓ 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 three 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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader;

black flat-bottom uncoated 96-well plates, fluorescence plate reader. For milk and solid samples, 0.45 μ m PTFE syringe filter and 5% isopropanol, 5% Triton X-100 solution.

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 0.45 μ m PTFE syringe filter (e.g. VWR Cat# 28145-493).

Note: SH-containing reagents (e.g. β -mercaptoethanol, dithiothreitol, > 5 μ M), 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 μ M stock of standard by diluting 5 μ L of the 100 mM Standard with 495 μ L Assay Buffer. Dilute the 1000 μ M standard in Assay Buffer as follows:

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

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

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

3. ACS reaction. Prepare enough ACS Working Reagent by mixing, for each well, 40 μ L Assay Buffer, 1 μ L Enzyme A, 5 μ L Substrate, 1 μ L ATP. Add 40 μ L 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 μ L Assay Buffer, 1 μ L Enzyme B and 1 μ L Dye Reagent. Add 50 μ L ACOD Working Reagent to each well. Tap plate to mix. Incubate 30 min at room temperature protected from light.
5. 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 μ M Standards and (2) a black 96-well plate are used. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm.

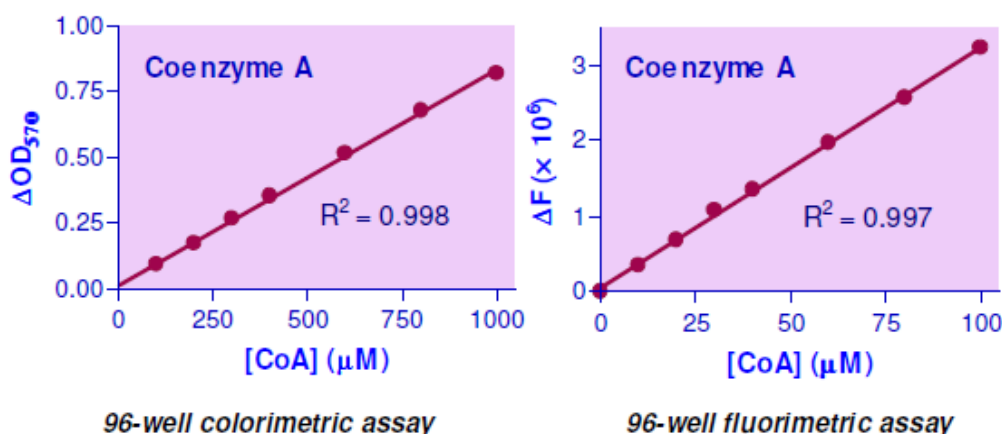
CALCULATION

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,

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

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 .



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.