Coenzyme A (CoA) Assay Kit (Colorimetric/Fluorometric)

LS-K182-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

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. LSBio's 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 H2O2. 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, fluorometric 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.

Components

Component	K182-100
	100 Tests
Assay Buffer	20 mL
Dye Reagent	120 µL
Enzyme A	Dried
Enzyme B	120 µL
Substrate	600 µL
Standard	50 µL
ATP	120 µL

Materials Not Supplied

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.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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Assay Procedure

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 flatbottom 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.
5. Read optical density at 570nm (550-585nm).

Fluorometric Assay

The fluorometric 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 λex = 530 nm and λ em = 585 nm.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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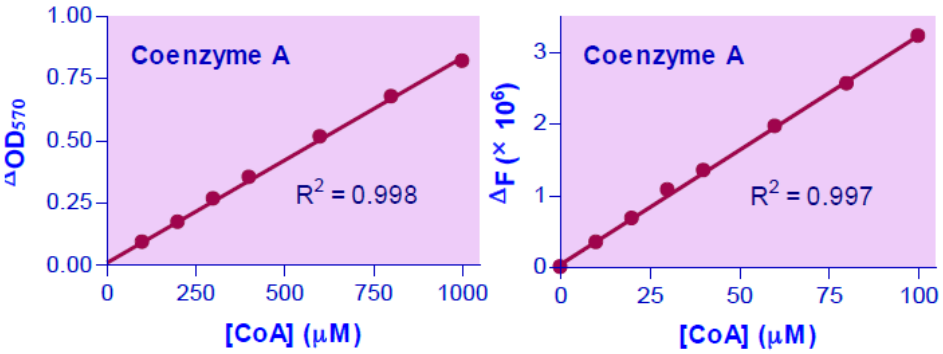
Calculations

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, 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 Fluorometric Assay, dilute sample in Assay Buffer and repeat the assay. Multiply result by the dilution factor, *n*.

Sample Data



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

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