Coenzyme A Assay Kit

(Colorimetric)

Coenzyme A Assay Kit (Colorimetric) is a detection kit for the quantification of Coenzyme A in Biological samples.

Catalog number: ARG82146

Package: 100 tests

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INTRODUCTION

Coenzyme A (CoA, SHCoA, CoA SH) is a coenzyme, notable for its role in the synthesis and oxidation of fatty acids, and the oxidation of pyruvate in the citric acid cycle. All genomes sequenced to date encode enzymes that use coenzyme A as a substrate, and around 4% of cellular enzymes use it (or a thioester) as a substrate. In humans, CoA biosynthesis requires cysteine, pantothenate (vitamin B_5), and adenosine triphosphate (ATP).

In its acetyl form, coenzyme A is a highly versatile molecule, serving metabolic functions in both the anabolic and catabolic pathways. Acetyl-CoA is utilised in the post-translational regulation and allosteric regulation of pyruvate dehydrogenase and carboxylase to maintain and support the partition of pyruvate synthesis and degradation. [Provide by Wikipedia: Coenzyme A]

PRINCIPLE OF THE ASSAY

This Coenzyme A Assay Kit (Colorimetric) is a simple, two step assay that measures the amount of coenzyme A present in biological samples. 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 570 nm or fluorescence intensity (ex 530 nm / em 585 nm) is directly proportional to the CoA concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
Enzyme A (lyophilized)	1 vial	-20°C
Enzyme B	120 µL	-20°C
Substrate	600 µL	-20°C
ATP	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard	50 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm.
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- 0.45 µm PTFE syringe filter
- 5% isopropanol and 5% Triton X-100 solution
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- 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.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Milk and solid samples: homogenized in 5% isopropanol and 5% Triton X-100 in distilled water, followed by filtration through a 0.45µm PTFE syringe filter.

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.

REAGENT PREPARATION

- **Enzyme A:** reconstitute Enzyme A by adding 120 µL of distilled water to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down and incubate at RT for 15 minutes. Store reconstituted Enzyme A at -20°C and use within 2 months.
- **ACS Working Reagent:** for each well, mixing 40 µL of Assay Buffer, 1 µL of Enzyme A, 5 µL of Substrate and 1 µL of ATP.
- **ACOD Working Reagent:** for each well, mixing 55 µL of Assay Buffer, 1 µL of Enzyme B, 1 µL of Dye Reagent.
- **Standard:** Prepare a 1000 µM stock of standard by diluting 5 µL of the 100

mM Standard with 495 µL of Assay Buffer. Dilute the 1000 µM standard in Assay Buffer as follows:

Standard tube	Coenzyme A (µM)	Assay Buffer (µL)	1000 µM Standard (µL)
S1	1000	0	100
S2	600	40	60
S3	300	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

Colorimetric Assay

1. Add **10 µL** of diluted **Standards** or **samples** into wells of clear bottom 96-well microplate.
2. Add **40 µL** of **ACS Working Reagent** to each well. Tap lightly to mix and incubate for **30 minutes** at **room temperature**.
3. Add **50 µL** of **ACOD Working Reagent** to each well. Tap lightly to mix and incubate for **30 minutes** at **room temperature** in the dark.
4. Read the absorbance at **O.D. 570 nm** (**550-585 nm**)

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 microplate are used. Read fluorescence intensity at λex = 530 nm and λ em = 585 nm.

CALCULATION OF RESULTS

1. Subtract blank value (S4, Assay Buffer) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope using linear regression fitting. Coenzyme A of the sample is calculated as follow:

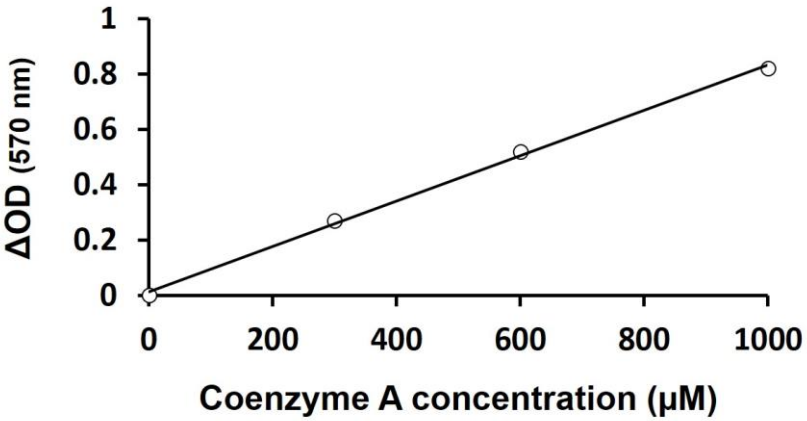
   CoA (µM) = [(R_{SAMPLE} – R_{BLANK}) / Slope] x n

   Note:

   - R_{SAMPLE}, R_{BLANK}: the O.D. 610 nm values or fluorescence intensity of the sample and blank.
   - 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.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Coenzyme A Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

3 µM