Technical Bulletin

Coenzyme A Assay Kit

Catalog number MAK504

Product 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.

The 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 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 at λ_{Ex} =530 nm/λ_{Em} =585 nm is directly proportional to the CoA concentration in the sample.

The linear detection range of the kit is 5 to 1000 µM Coenzyme A for the colorimetric assay and 3 to 100 µM for the fluorometric assay. The kit is suitable for Coenzyme A activity determination in a variety of biological samples.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalog Number MAK504A
- Dye Reagent 120 µL
  Catalog Number MAK504B
- Enzyme A 1 vial
  Catalog Number MAK504C
- Enzyme B 120 µL
  Catalog Number MAK504D
- Substrate 600 µL
  Catalog Number MAK504E
- ATP 120 µL
  Catalog Number MAK504F
- Standard (100 mM) 50 µL
  Catalog Number MAK504G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example., multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

For Milk and Solid Samples Only

- 0.45 µm PTFE syringe filter
  (Catalog Number SLHNX13 or equivalent)
- 5% isopropanol
  (Catalog Number 190764 or equivalent)
- Triton™ X-100
  (Catalog Number X100 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. Keep thawed tubes on ice during assay. Important: the thawed Standard solution should be clear and colorless. If the Standard is turbid, bring it to 37 °C and gently swirl the tube (do not vortex) until the solution is clear.

Enzyme A

Reconstitute vial with 120 µL of purified water. Make sure Enzyme A is fully dissolved by pipetting up and down and incubate at room temperature for 15 minutes. Store reconstituted Enzyme A at -20 °C and use within 2 months of reconstitution.

Sample Preparation

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

- 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 purified water, followed by filtration through a 0.45 µm PTFE syringe filter.

Transfer 10 µL of each Sample into separate wells of the appropriate plate.

Colorimetric Standard Curve Preparation

1. Prepare a 1000 µM standard by diluting 5 µL of 100 mM Standard with 495 µL of Assay Buffer.
2. Dilute standards in Assay Buffer according to Table 1.

Table 1.
Preparation of Colorimetric Coenzyme A Standards

Well No.	1000 µM Standard	Assay Buffer	Coenzyme A (µM)
1	100 µL	0 µL	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	0 µL	100 µL	0

3. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 µL of the standards from Colorimetric Procedure with 90 µL of Assay Buffer according to Table 2.

Table 2.
Preparation of Fluorometric Coenzyme A Standards

Well No.	Colorimetric Standard	Assay Buffer	Coenzyme A (µM)
1	10 µL of 1000 µM Std	90 µL	100
2	10 µL of 600 µM Std	90 µL	60
3	10 µL of 300 µM Std	90 µL	30
4	-	100µL	0

3. Mix well and transfer 10 µL of each Standard into separate wells of a black 96-well plate

Working Reagent Preparation

Acyl CoA Synthetase (ACS) Reaction

1. Mix enough ACS Working Reagent for the number of assays to be performed. For each well, prepare 47 µL of ACS Working Reagent according to Table 3.

Table 3.
Preparation of ACS Working Reagent

Reagent	Volume
Assay Buffer	40 µL
Enzyme A	1 µL
Substrate	5 µL
ATP	1 µL

2. Transfer 40 µL of ACS Working Reagent into each well. Tap plate to mix.
3. Incubate the plate at room temperature for 30 minutes.

Acyl CoA Oxidase (ACOD) Reaction

1. Mix enough ACOD Working Reagent for the number of assays to be performed. For each well, prepare 57 µL of ACOD Working Reagent according to Table 4.

Table 4.
Preparation of ACOD Working Reagent

Reagent	Volume
Assay Buffer	55 µL
Enzyme B	1 µL
Dye Reagent	1 µL

2. Transfer 50 µL of ACOD Working Reagent into each well. Tap plate to mix.
3. Incubate the plate at room temperature for 30 minutes protected from light.

Measurement

Measure the optical density (OD) at 570 nm or fluorescence intensity (F) at λ_{Ex} = 530 nm/λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔF or ΔOD against the standard concentrations and determine the slope of the standard curve.
3. Calculate the Coenzyme A concentration of Samples using the below equation:
4.

$$\text{CoA (µM)} = \frac{R_{Sample} - R_{Blank}}{\text{Slope}} \times DF$$

where:

R_{Sample} = OD or fluorescence intensity (F) reading of Sample

R_{Blank} = OD or fluorescence intensity (F) reading of Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated CoA concentration of a sample is higher than 1000 µM for 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 (DF).

Figure 1.

Typical Colorimetric Coenzyme A Standard Curve

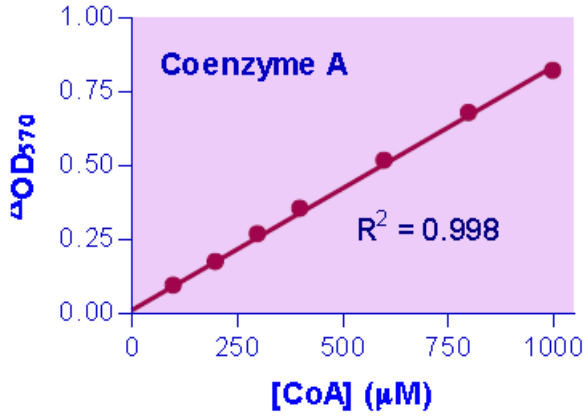
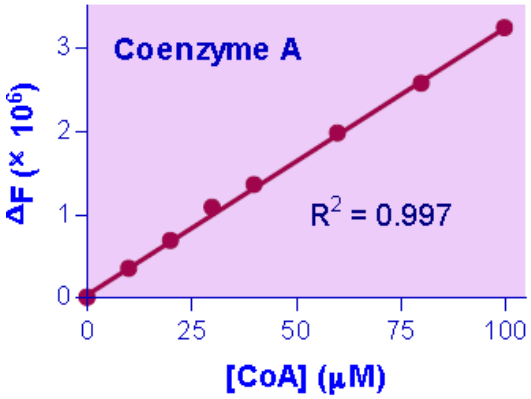


Figure 2.
Typical Fluorometric Coenzyme A Standard Curve



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