Amplite™ Fluorimetric Coenzyme A Quantitation Kit *Green Fluorescence*

Catalog number: 15270
Unit size: 200 Tests

Component	Storage	Amount
Component A: CoA Green™	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component C: Coenzyme A (CoA) Standard (FW=767.53)	Freeze (<-15 °C), Minimize light exposure	1 vial (154 µg)
Component D: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

Coenzyme A (CoA) is a universal and essential cofactor in all forms of cellular life acting as a principal acyl carrier in numerous biosynthetic, energy-yielding, and degradative pathways. It plays important roles in the synthesis and oxidation of fatty acids, pyruvate oxidation and the citric acid cycle. Measurement of CoA is one of the essential tasks for investigating biological processes and events in many biological systems. There are a few reagents or assay kits available for quantitating CoA content in biological systems. However, the existing commercial kits either lack sensitivity or have tedious procedures. Our Amplite™ Fluorimetric CoA Qutitation Assay Kit provides an ultrasensitive fluorimetric assay to quantitate CoA content by detection of -SH group in CoA. Our proprietary fluorogenic CoA Green™ dye used in the kit becomes strongly fluorescent upon reacting with -SH. The assay kit can detect as little as 4 picomole of CoA in a 100 µL assay volume (40 nM). It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

AT A GLANCE

Protocol summary

1. Prepare CoA working solution (50 µL)
2. Add CoA standards or test samples (50 µL)
3. Incubate at RT for 10 minutes - 1 hour
4. Monitor the fluorescence increase at Ex/Em = 490/520 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	520 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. CoA standard solution (1 mM):
Add 200 µL of ddH_2O into the CoA standard vial (Component C) to make 1 mM (1 nmol/µL) stock solution.

Note It is highly recommended to use the ddH_2O that has been sparged with nitrogen to remove oxygen for preparing coenzyme A standard solution. The aqueous solution is not stable and will degrade rapidly. It should be stored at 2 - 8 °C and used within the day.

2. CoA Green™ stock solution (100X):
Add 100 µL of DMSO (Component D) into the vial of CoA Green™ (Component A) to make 100X stock solution.

PREPARATION OF STANDARD SOLUTION

CoA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15270

Add 30 µL of CoA standard solution to 970 µL of Assay Buffer (Component B) to generate 30 µM (30 pmol/µL) CoA standard. Take the 30 µM CoA standard solution to perform 1:3 serial dilutions with Assay buffer (Component B) to get serial dilutions of CoA standard (CoA1 - CoA7).

Note Diluted CoA standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 50 µL of CoA Green™ stock solution (100X) into 5 mL of Assay Buffer (Component B) and mix well.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of CoA standards and test samples in a solid black 96-well microplate. CoA = Coenzyme A standard (CoA1 - CoA7, 0.01 to 10 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
CoA1	CoA1	...	...
CoA2	CoA2	...	...
CoA3	CoA3		
CoA4	CoA4		
CoA5	CoA5		
CoA6	CoA6		
CoA7	CoA7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
CoA1-CoA7	50 µL	serial dilution (0.01 to 10 µM)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	sample

1. Prepare coenzyme A standards (CoA), blank controls (BL), and test samples (TS) according ot the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of CoA working solution to each well of the CoA standard, blank control, and test sample to make the total CoA assay volume of 100 µL/well. For a 384-well plate, add 25 µL of CoA working solution into each well instead,

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for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 minutes to 1 hour, protected from light.

4. Monitor the fluorescence increase at Ex/Em = 490/520 nm with a fluorescence plate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Coenzyme A samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

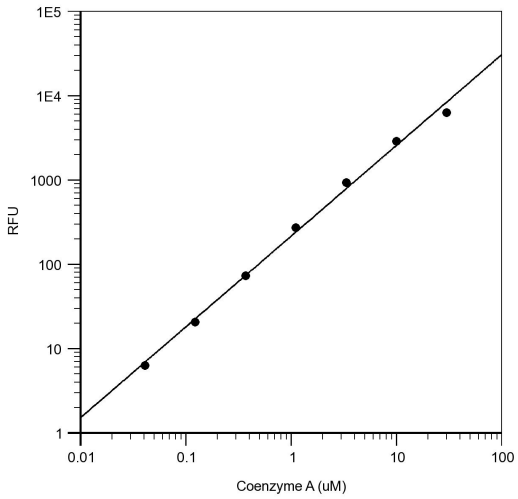

Figure 1. CoA dose response was measured in a 96-well solid black plate with Amplite™ Fluorimetric Coenzyme A Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech).

DISCLAIMER