Amplite™ Fluorimetric Coenzyme A Quantitation Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 15270 (100 assays)	Keep at -20 °C Avoid exposure to moisture and light	Fluorescence microplate readers

Introduction

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. 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 at Ex/Em = 490/520 nm, and easily adapted to automation without a separation step.

Kit Components

Components	Amount
Component A: CoA Green™	1 vial
Component B: Assay Buffer	1 bottle (25 mL)
Component C: Coenzyme A (CoA) Standard (FW=767.53)	1 vial (154 µg)
Component D: DMSO	1 vial (200 µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare CoA assay mixture (50 µL) → Add CoA standards or test samples (50 µL) → Incubate at RT for 10 minutes - 1 hour → Monitor the fluorescence increase at Ex/Em = 490/520 nm

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

1. Prepare CoA standard stock solution:

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

Note1: It is highly recommended to use the ddH_2O that has been sparged with nitrogen to remove oxygen for preparing coenzyme A stock solution.

Note2: The aqueous solution is not stable, will degrade rapidly. It should be stored at 2-8°C and used within 1 day.

2. Prepare 100X CoA Green™ stock solution:

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

Note: The unused CoA Green™ stock solution should be divided into single use aliquots, stored at -20°C and kept from light.

3. Prepare CoA Assay mixture:

Add 50 µL of 100X CoA Green™ stock solution (from Step 2) into 5 mL of Assay Buffer (Component B), and mix them well.

4. Prepare serial dilutions of CoA standard (0 to 30 µM):

4.1 Add 30 µL of CoA standard stock solution (from Step 1) to 970 µL of Assay Buffer (Component B) to generate 30 µM (30 pmol/µL) CoA standard.
Note: Diluted CoA standard solution is unstable, and should be used within 4 hours.

4.2 Take 200 µL of 30 µM CoA standard solution to perform 1:3 serial dilutions with Assay buffer (Component B) to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 µM serial dilutions of CoA standard.

4.3 Add CoA standards and CoA-containing test samples into a solid black 96-well microplate as shown in Tables 1 and 2.

Note: Treat cells or tissue samples as desired.

Table 1: Layout of CoA standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
CoA1	CoA 1	….	….	….	….						
CoA 2	CoA 2										
CoA 3	CoA 3										
CoA 4	CoA 4										
CoA 5	CoA 5										
CoA 6	CoA 6										
CoA 7	CoA 7										

Note: CoA = CoA Standards, BL=Blank Control, TS=Test Samples.

Table 2: Reagent composition for each well

CoA Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

**Note: Add the serial dilutions of CoA standard from 0.01 µM to10 µM into wells from CoA 1 to CoA 7 in duplicate.*

5. Run CoA assay:

5.1 Add 50 µL of CoA reaction mixture (from Step 3.1) to each well of the CoA standard, blank control, and test samples (see Step 4.3) to make the total CoA assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of CoA reaction mixture into each well.

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

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

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the CoA reactions. A CoA standard curve is shown in Figure 1.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

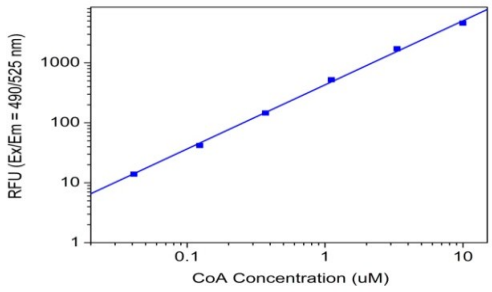

Figure 1 CoA dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Coenzyme A Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech). As low as 40 nM (4 pmol/well) of CoA was detected with 30 minutes incubation time (n=3).

References

1. Begley, T. P., C. Kinsland, and E. Strauss. 2001. The biosynthesis of coenzyme A in bacteria. Vitam Horm 61: 157-71.
2. Daugherty, M., B. Polanuyer, M. Farrell, M. Scholle, A. Lykidis, V. de Crecy-Lagard, and A. Osterman. 2002. Complete reconstitution of the human coenzyme A biosynthetic pathway via comparative genomics. J Biol Chem 277:21431-9.
3. Genschel, U. 2004. Coenzyme A Biosynthesis: Reconstruction of the Pathway in Archaea and an Evolutionary Scenario Based on Comparative Genomics. Mol Biol Evol.