**Amplite™ Fluorimetric Coenzyme A Quantitation Kit**

*Green Fluorescence*

### 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 Quantitation 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

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Component A: CoA Green™</td>
<td>1 vial</td>
</tr>
<tr>
<td>Component B: Assay Buffer</td>
<td>1 bottle (25 mL)</td>
</tr>
<tr>
<td>Component C: Coenzyme A (CoA) Standard (FW=767.53)</td>
<td>1 vial (154 μg)</td>
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<tr>
<td>Component D: DMSO</td>
<td>1 vial (200 μL)</td>
</tr>
</tbody>
</table>

### 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 ddH₂O into the CoA standard vial (Component C) to make 1 mM (1 nmol/μL) stock solution.
   *Note 1: It is highly recommended to use the ddH₂O that has been sparged with nitrogen to remove oxygen for preparing coenzyme A stock solution.*
   *Note 2: 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):**
   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.*
   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.
   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

<table>
<thead>
<tr>
<th>BL</th>
<th>BL</th>
<th>TS</th>
<th>TS</th>
<th>....</th>
<th>....</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoA 1</td>
<td>CoA 1</td>
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<tr>
<td>CoA 2</td>
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<td>CoA 3</td>
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<tr>
<td>CoA 7</td>
<td>CoA 7</td>
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</tbody>
</table>

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

Table 2: Reagent composition for each well

<table>
<thead>
<tr>
<th>CoA Standard</th>
<th>Blank Control</th>
<th>Test Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Dilutions*: 50 μL</td>
<td>Assay Buffer: 50 μL</td>
<td>50 μL</td>
</tr>
</tbody>
</table>

*Note: Add the serial dilutions of CoA standard from 0.01 μM to 10 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](image)

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


Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.