ab138889 –

Coenzyme A Assay Kit

(Fluorometric - Green)

Instructions for Use

This kit provides an ultrasensitive fluorometric assay to quantitate CoA content in biological systems.

This product is for research use only and is not intended for diagnostic use.

Version 3 Last updated 20 May 2020

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1. 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.

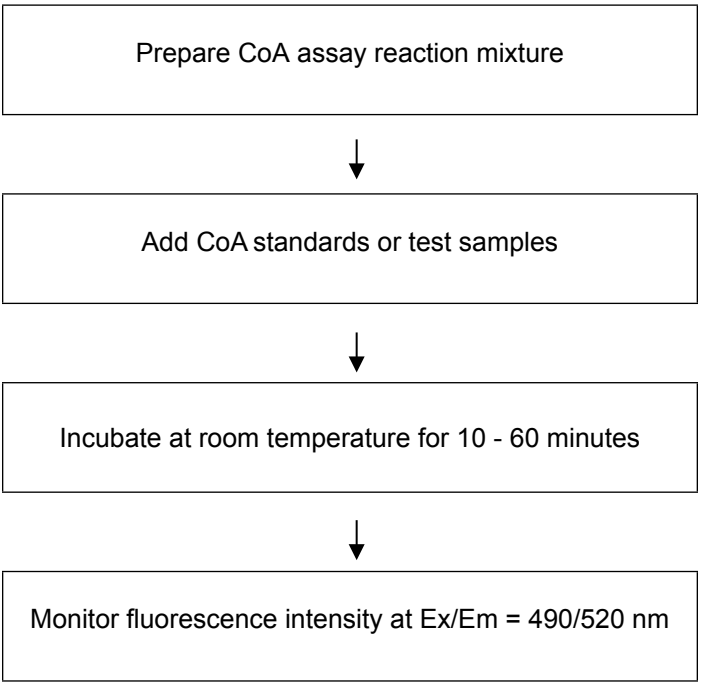
There are a few reagents or assay kits available for quantitating CoA content in biological systems. The existing commercial kits either lack sensitivity or have tedious procedures.

ab138889 provides an ultrasensitive fluorometric assay to quantitate CoA content by detection of –SH group in CoA. Our proprietary fluorogenic CoA green Indicator dye used in the kit becomes strongly fluorescent upon reacting with –SH.

ab138889 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.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

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

4. Storage and Handling

Keep at -20°C. Avoid exposure to moisture and light.

5. Additional Materials Required

- 96 or 384-well solid, black microplates: Tissue culture microplates with white wall and clear bottom
- Fluorescence microplate reader
- ddH_2O

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

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

Note: The unused CoA solution should be divided into single use aliquots and stored at -20˚C.

B. Prepare 100X CoA green Indicator stock solution: Add 100 µL of DMSO (Component D) into the vial of CoA green Indicator (Component A) to make 100X stock solution.

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

C. Prepare CoA Assay reaction mixture: Add 50 µL of 100X CoA green Indicator stock solution (from Step B) into 5 mL of Assay Buffer (Component B) and mix them well.

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

1. Add 30 µL of CoA standard stock solution (Step A) to 970 µL of Assay Buffer (Component B) to generate 30 µM (30 pmol/µL) CoA standard solution.

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 generate 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 µM serial dilutions of CoA standard.

3. Add serially diluted CoA standards and/or CoA-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Note: Treat cells or tissue samples as desired.

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

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

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

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

Table 2. Reagent composition for each well.

**Note: Add the serial dilutions of CoA standard from 0.01 to 10 µM into wells from CoA1 to CoA7 in duplicate.*

E. Run CoA assay:

1. Add 50 µL of CoA assay reaction mixture (Step C) to each well of the CoA standard, blank control, and test samples 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 in each well.

2. Incubate the reaction for 10 to 60 minutes at room temperature, protected from light.

3. Monitor the absorbance increase with a fluorescence microplate reader at Ex/Em = 490/520 nm.

7. Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and 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.

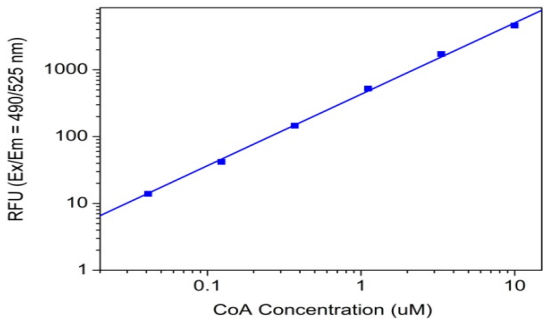


Figure 1. CoA dose response was measured in a 96-well black plate with Coenzyme A Assay Kit using a microplate reader. As low as 40 nM (4 pmol/well) of CoA was detected with 30 minutes incubation time (n=3).

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

For all technical and commercial enquires please go to:
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