Coenzyme A Assay Kit

Item No. 700440

Customer Service 800.364.9897 * **Technical Support** 888.526.5351

www.caymanchem.com

TABLE OF CONTENTS

Materials Supplied

Item Number	Item	Quantity/Size	Storage
700441	Coenzyme A Standard	1 vial/5 mg	-20°C
700442	CoA Periodic Acid	2 vials	-20°C
700443	CoA Ammonium Acetate	1 vial/6 ml	-20°C
700444	CoA Europium Chloride	2 vials	-20°C
700445	CoA Tetracycline	2 vials	-20°C
400013	96-Well Solid Plate (white)	1 plate	Room temperature
400012	96-Well Cover Sheet	1 cover	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength between 380-390 nm and an emission wavelength between 610-615 nm
2. Adjustable pipettors and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Acetonitrile - A.C.S. Grade for chromatographic and UV spectrophotometric use

INTRODUCTION

Background

Coenzyme A (CoA) is biosynthesized in a five-step enzymatic process from pantothenate (Vitamin B_5).[1] CoA is an indispensible cofactor in all living organisms, functioning as an acyl group carrier and carbonyl-activating group in a number of key biochemical reactions, including the TCA cycle and fatty acid metabolism. CoA is involved in over 100 different reactions in intermediary metabolism with approximately 4% of known enzymes utilizing CoA as a cofactor.[2,3] One of the primary functions of CoA is carrying and transferring acyl groups, with the most important being the acetate group, forming the molecule acetyl-CoA. This acetyl group eventually becomes incorporated in molecules such as cholesterol, acetylcholine, melatonin, heme, and intermediates in the TCA cycle.[4] In rat liver, intracellular levels of CoA increase in conditions such as diabetes, fasting, treatment with hypolipidemic drugs, and consuming a high fat diet.[5] Animal models show low CoA levels in tumors.[6,7]

About This Assay

Cayman's Coenzyme A Assay Kit provides a simple, reproducible, and sensitive tool for assaying coenzyme A from plasma, serum, urine, cell lysates, and tissue homogenates. Coenzyme A forms a complex with europium chloride and tetracycline in the presence of periodic acid. This complex can be analyzed with an excitation wavelength between 380-390 nm and an emission wavelength between 610-615 nm.

$$\text{CoA + Europium Chloride + Tetracycline} \xrightarrow{\text{Periodic Acid}} (\text{CoA-Eu-TC})^{3+} \quad (Ex_{385}/Em_{612})$$

PRE-ASSAY PREPARATION

Reagent Preparation

1. Coenzyme A Standard - (Item No. 700441)

The vial contains 5 mg of Coenzyme A that will be used to prepare the standard curve (see **Standard Preparation** on page 11).

2. CoA Periodic Acid - (Item No. 700442)

Each vial contains a lyophilized powder of periodic acid. Reconstitute the contents of one vial with 4 ml of HPLC-grade water. One vial is enough reagent to assay 60 wells. If additional wells are being utilized, then reconstitute the contents of the second vial. The reconstituted solution is stable for eight hours at room temperature.

3. CoA Ammonium Acetate - (Item No. 700443)

The vial contains 6 ml of 100 mM Ammonium Acetate, pH 6.8. Thaw the vial at room temperature. The vial is ready to use as supplied. When stored at 4°C, ammonium acetate is stable for at least six months.

4. CoA Europium Chloride - (Item No. 700444)

Each vial contains a lyophilized powder of europium chloride. Reconstitute the contents of one vial with 3 ml of HPLC-grade water. One vial is enough reagent to assay 75 wells. If additional wells are being utilized, then reconstitute the contents of the second vial. The reconstituted solution is stable for one hour at room temperature.

5. CoA Tetracycline - (Item No. 700445)

Each vial contains a lyophilized powder of tetracycline. Reconstitute the contents of one vial with 3 ml of HPLC-grade water. One vial is enough reagent to assay 60 wells. If additional wells are being utilized, then reconstitute the contents of the second vial. The reconstituted solution is stable for one hour at room temperature.

6. Acetonitrile/Water Solution

Prepare a stock acetonitrile/water solution by adding 500 µl of HPLC-grade water and 1 ml of acetonitrile and mix until homogeneous. To a separate tube, add 9 ml of HPLC-grade water and 1 ml of the freshly prepared acetonitrile/water solution and mix until homogeneous. Use this 1:10 acetonitrile/water solution to prepare the standard curve (see **Standard Preparation** on page 11) and to dilute samples if necessary.

Sample Preparation

Plasma

1. Collect blood using an anticoagulant such as heparin, EDTA, or sodium citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice.
3. Add 1 ml of acetonitrile to a 1.5 ml micro-centrifuge tube. Add 500 µl of the collected plasma and vortex.
4. Centrifuge at 10,000 x g for 15 minutes at 4°C.
5. Collect the supernatant and store on ice. At this point, if not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
6. Typically, plasma samples require dilutions of at least 1:10 or greater prior to measurement in the assay. Dilute the plasma samples using the 1:10 acetonitrile/water solution before assaying.

Serum

1. Collect blood without using an anticoagulant.
2. Allow the blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice.
4. Add 1 ml of acetonitrile to a 1.5 ml micro-centrifuge tube. Add 500 µl of the collected serum and vortex.
5. Centrifuge at 10,000 x g for 15 minutes at 4°C.
6. Collect the supernatant and store on ice. At this point, if not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
7. Typically, serum samples require dilutions of at least 1:10 or greater prior to measurement in the assay. Dilute the serum samples using the 1:10 acetonitrile/water solution before assaying.

Urine

1. Collection of urine does not require any special treatment.
2. Add 1 ml of acetonitrile to a 1.5 ml micro-centrifuge tube. Add 500 µl of urine and vortex.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Collect the supernatant and store on ice. At this point, if not assaying the same day, freeze at -80°C. The urine sample will be stable for one month while stored at -80°C.
5. Typically, urine samples require dilutions of at least 1:10 or greater prior to measurement in the assay. Dilute the urine samples using the 1:10 acetonitrile/water solution before assaying.

NOTE: CoA values from urine samples can be standardized using Cayman's Creatinine Assay Kit (Item No. 500701).

Cell Lysates

1. Centrifuge cells at 700-1,000 x g for 10 minutes at 4°C. Pipette off the supernatant and discard.
2. Resuspend cell pellet in 5 ml of PBS and centrifuge at 700-1,000 x g for 10 minutes at 4°C to remove any residual medium. Pipette off the supernatant and discard.
3. Resuspend cell pellet in 1-2 ml of cold PBS. Store cells on ice.
4. Sonicate the cell suspension 20X at one second bursts.
5. Centrifuge the cell suspension at 10,000 x g for 10 minutes at 4°C.
6. Aliquot the supernatant into a vial and freeze at -80°C until use. The sample will be stable for one month.
7. Add 500 µl of acetonitrile to a micro-centrifuge tube. Add 250 µl of the cell lysate and vortex.
8. Centrifuge at 10,000 x g for 15 minutes at 4°C.
9. Collect the supernatant and store on ice.
10. If necessary, dilute the cell lysate samples using the 1:10 acetonitrile/water solution before assaying.

Tissue Homogenates

1. Weigh tissue and then mince into small pieces.
2. Homogenize 350-400 mg of minced tissue in 2 ml of a buffer of choice.
3. Centrifuge the homogenized tissue at 700-1,000 x g for 10 minutes at 4°C.
4. Collect the supernatant in a separate tube. If not assaying on the same day, freeze the supernatant at -80°C until use. The sample will be stable for one month.
5. Add 500 µl of acetonitrile to a micro-centrifuge tube. Add 250 µl of the tissue homogenate and vortex.
6. Centrifuge at 10,000 x g for 15 minutes at 4°C.
7. Collect the supernatant and store on ice.
8. If necessary, dilute the tissue homogenate samples using the 1:10 acetonitrile/water solution before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that each sample and standard be assayed at least in duplicate (triplicate is preferred). A typical layout of CoA standards and samples to be measured in duplicate is given below in Figure 1. We suggest you record the contents of each well on the template sheet provided (see page 19).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H = Standards
S1-S40 = Sample Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 250 µl in all wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate (triplicate is preferred), but it is the user's discretion to do so.
- The assay is performed at room temperature (25°C).
- Monitor the fluorescence with an excitation wavelength between 380-390 nm and an emission wavelength between 610-615 nm.

Standard Preparation

In a separate vial, weigh out 1 mg of coenzyme A. Add 1.3 ml of water to obtain a 1 mM Stock Solution. Take eight clean test tubes and label them A-H. Add the amount of 1 mM Coenzyme A Stock Solution and the 1:10 Acetonitrile/Water Solution to each tube as described in Table 1. We recommend that you store these diluted standards for no more than one hour. The 1 mM stock solution is stable for five days when stored at -20°C.

Tube	1 mM Coenzyme A (µl)	1:10 Acetonitrile/Water (µl)	Coenzyme A Concentration (µM)
A	0	1,000	0
B	5	995	5
C	10	990	10
D	20	980	20
E	40	960	40
F	60	940	60
G	80	920	80
H	100	900	100

Table 1. Preparation of Coenzyme A Standard Curve

Performing the Assay

1. **Standard Wells** - Add 50 µl of standard (tubes A-H) per well in the designated wells on the plate (see **Sample Plate Format**, Figure 1, page 10).
2. **Sample Wells** - Add 50 µl of the sample to at least two wells. To obtain reproducible results, the amount of coenzyme A added to the wells should fall within the range of the assay. When necessary, samples should be diluted with 1:10 acetonitrile/water solution.
3. Add 60 µl of CoA Periodic Acid (Item No. 700442) to standard and sample wells.
4. Add 50 µl of CoA Ammonium Acetate (Item No. 700443) to standard and sample wells.
5. Add 40 µl of CoA Europium Chloride (Item No. 700444) to standard and sample wells.
6. Add 50 µl of CoA Tetracycline (Item No. 700445) to standard and sample wells.

Well	Standard (µl)	Sample (µl)	Periodic Acid (µl)	Ammonium Acetate (µl)	Europium Chloride (µl)	Tetracycline (µl)
Standard	50	0	60	50	40	50
Sample	0	50	60	50	40	50

7. Cover the plate with the plate cover, and incubate for 25 minutes at room temperature.
8. Remove the plate cover and read using an excitation wavelength between 380-390 nm and an emission wavelength between 610-615 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence of each standard and sample.
2. Determine the average fluorescence of the standards. Subtract the fluorescence value of standard A (0 µM) from itself and all other standards. This is the corrected fluorescence.
3. Subtract the fluorescence value of standard A (0 µM) from the average value of each sample to yield the corrected sample fluorescence (CSF).
4. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of the final concentration of coenzyme A from Table 1. See Figure 2, on page 15, for a typical standard curve.
5. Calculate the coenzyme A concentration of the samples using the equation obtained from the linear regression of the standard curve, substituting the corrected sample fluorescence (CSF) for each sample.

$$\textbf{Coenzyme A Concentration (µM)} = \left[\frac{\text{CSF - (y-intercept)}}{\text{Slope}}\right] \text{ x Sample dilution}$$

Performance Characteristics

Precision:

When a series of 96 serum and urine measurements were performed on the same day, the intra-assay coefficient of variation was 3.2% and 3.7%, respectively. When a series of 48 serum and urine measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 2.6% and 3.1%, respectively.

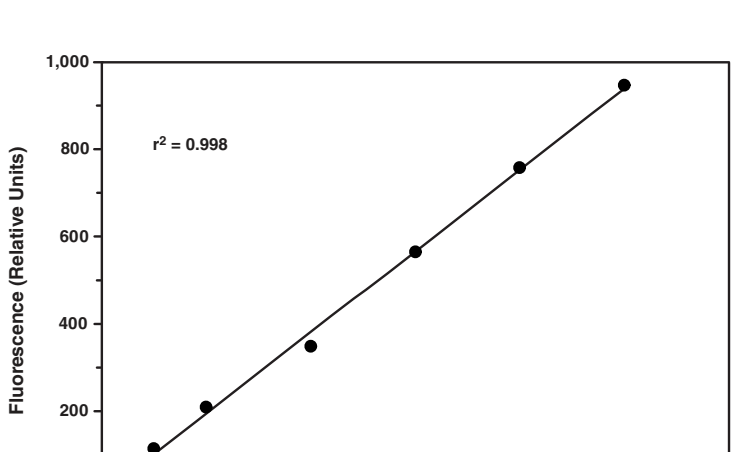


Figure 2. Coenzyme A standard curve

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Sample CoA Fluorescence was not above Standard A fluorescence	The CoA concentration is too low to detect	Re-assay the sample using a lower dilution
Sample CoA Fluorescence was above the highest point in the standard curve	The CoA concentration is too high in the sample	Dilute samples with the 1:10 acetonitrile/water solution and re-assay; *NOTE: Remember to account for the dilution factor when calculating CoA concentration.*
The fluorometer exhibited "MAX" values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read

References

1. Leonardi, R., Zhang, Y.-M., Rock, C.O., *et al.* Coenzyme A: Back in action. *Prog. Lipid Res.* **44(2-3)**, 125-153 (2005).
2. Begley, T.P., Kinsland, C., and Strauss, E. The biosynthesis of coenzyme A in bacteria. *Vitam. Horm.* **61**, 157-171 (2001).
3. Jackowski, S. Biosynthesis of pantothenic acid and coenzyme A., Chapter 44, *in Escherichia coli* and *Salmonella typhimurium*: Cellular and molecular biology. Neidhardt, F.C., Curtiss, R., Gross, C.A. *et al.*, editors, American Society for Microbiology, Washington D.C., 687-694 (1996).
4. Liu, G., Chen, J., Che, P., *et al.* Separation and quantitation of short-chain coenzyme A's in biological samples by capillary electrophoresis. *Anal. Chem.* **75**, 78-82 (2003).
5. Horie, S., Isobe, M., and Suga, T. Changes in CoA pools in hepatic peroxisomes of the rat under various conditions. *J. Biochem.* **99**, 1345-1352 (1986).
6. Rapp, G.W. Some systemic effects of malignant tumors. I. Co-enzyme A levels. *Cancer* **31(2)**, 357-360 (1973).
7. McAllister, R.A., Fixter, L.M., and Campbell, E.H.G. The effect of tumor growth on liver pantothenate, CoA, and fatty acid synthetase activity in the mouse. *Br. J. Cancer* **57**, 83-86 (1988).

Related Products

Adipogenesis Assay Kit - Item No. 10006908
Adipolysis Assay Kit - Item No. 10009381
ChREBP Cell-Based Translocation Assay Kit - Item No. 10010060
ChREBP Transcription Factor Assay Kit - Item No. 10006909
Creatine Kinase Fluorometric Assay Kit - Item No. 700630
Creatinine (urinary) Colorimetric Assay Kit - Item No. 500701
FABP4 Inhibitor/Ligand Screening Assay Kit - Item No. 10010231
Free Fatty Acid Fluorometric Assay Kit - Item No. 700310
Glucose Colorimetric Assay Kit - Item No. 10009582
Glycerol Colorimetric Assay Kit - Item No. 10010755
Glycogen Assay Kit - Item No. 700480
β-Hydroxybutyrate (Ketone Body) Colorimetric Assay Kit - Item No. 700190
LDL Uptake Cell-Based Assay Kit - Item No. 10011125
Lipid Droplets Fluorescence Assay Kit - Item No. 500001
Lipid Hydroperoxide (LPO) Assay Kit - Item No. 705002
Phosphatidylcholine Colorimetric Assay Kit - Item No. 10009926
PPARα, δ, γ Complete Transcription Factor Assay Kit - Item No. 10008878
PPARγ Ligand Screening Assay Kit - Item No. 10007685
SREBP-1 Transcription Factor Assay Kit - Item No. 10010854
SREBP-2 Cell-Based Translocation Assay Kit - Item No. 10009239
SREBP-2 Transcription Factor Assay Kit - Item No. 10007819
Steatosis Colorimetric Assay Kit - Item No. 10012643
Triglyceride Colorimetric Assay Kit - Item No. 10010303
For a complete list of related products please visit: www.caymanchem.com/catalog/700440

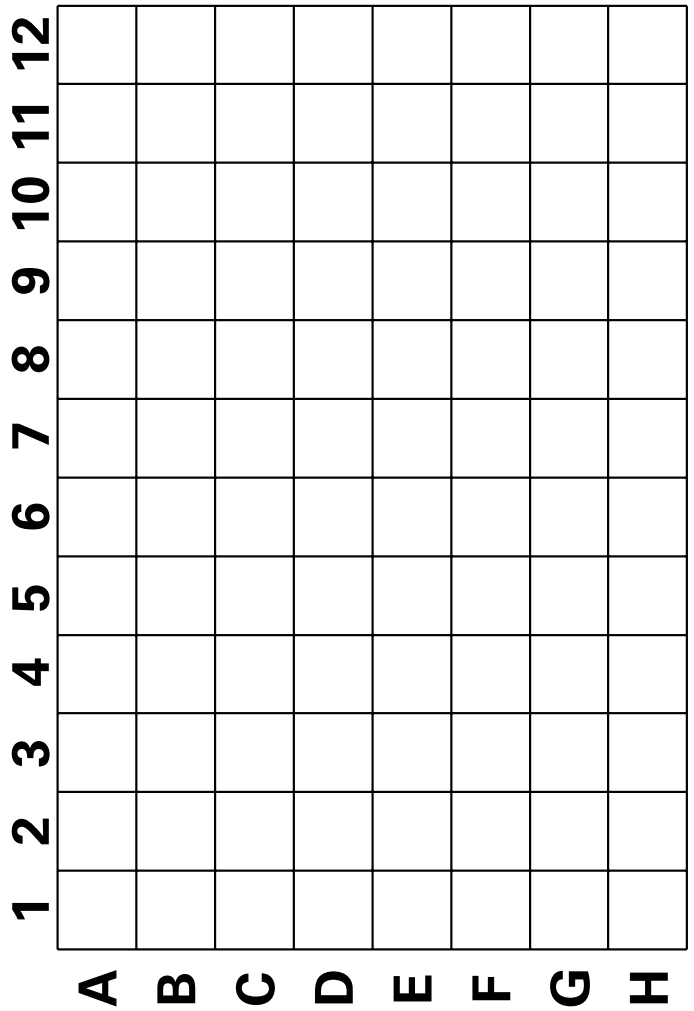
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



NOTES