ab87546
PicoProbe Acetyl CoA Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of acetyl CoA levels in various samples.

View kit datasheet: www.abcam.com/ab87546
(use www.abcam.cn/ab87546 for China, or www.abcam.co.jp/ab87546 for Japan)

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 15, Last Updated 24 October 2023

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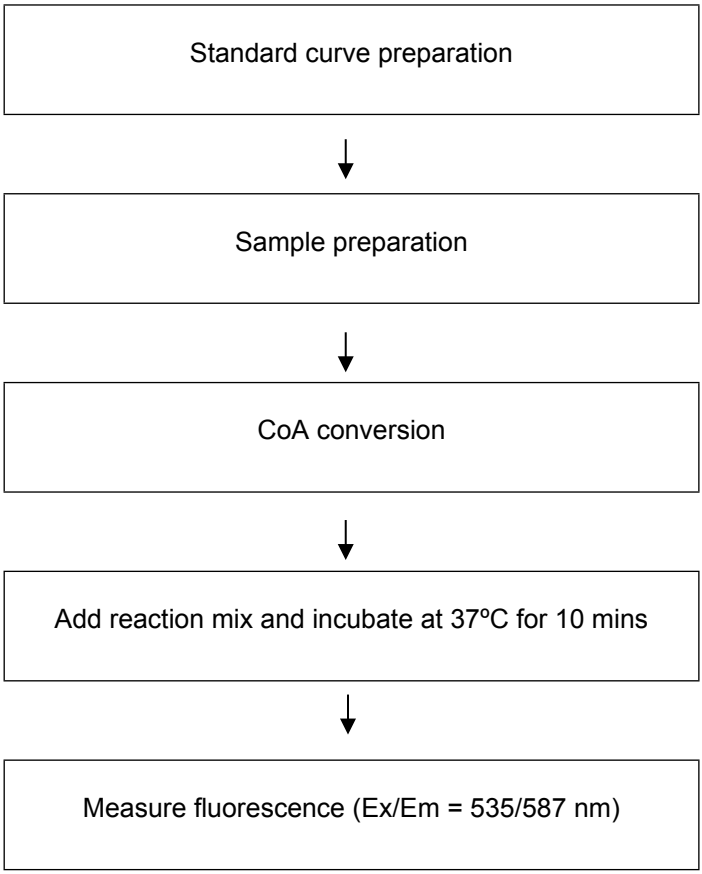
1. BACKGROUND

PicoProbe Acetyl CoA Assay Kit (fluorometric) (ab87546) is a highly sensitive assay for determining acetyl CoA level in a variety of biological samples. In the assay, free CoA is quenched then acetyl CoA is converted to CoA. The CoA is reacted to form NADH which interacts with PicoProbe II/PicoProbe to generate fluorescence (Ex=535 / Em=587 nm). The assay can detect 10-500 pmol of Acetyl CoA (with detection limit ~0.4 µM) in a variety of samples.

Acetyl CoA is a central molecule of metabolism. It carries acetate, used in the build-up and breakdown of larger molecules. Acetyl CoA is key in synthetic pathways leading to sesquiterpenes, precursors to cholesterol and other sterols, flavenoids and other polyketides, polyenes and long-chain fatty acids. It is the source of the acetyl group used in histone acetylation. The acetyl group is also incorporated into a variety of other molecules such as acetylcholine, melatonin, heme and TCA cycle intermediates.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer XXII/Acetyl CoA Assay Buffer	25 mL	-20°C	-20°C
PicoProbe II/PicoProbe	200 µL	-20°C	-20°C
Converter Enzyme III/Conversion Enzyme	100 µL	-20°C	-20°C
Acetyl CoA Enzyme Mix	500 µL	-20°C	-20°C
Acetyl CoA Substrate Mix (Lyophilized)	1 vial	-20°C	-20°C
CoA Quencher	1 mL	-20°C	-20°C
Quench Remover I/Quench Remover (Lyophilized)	1 vial	-20°C	-20°C
Acetyl CoA Standard (1 µmol, Lyophilized)	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- 96 well plate: black plates (clear bottoms)
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)
- 3M $KHCO_3$ (if using tissue)

If performing deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer XXII/Acetyl CoA Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **PicoProbe II/Pico Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light. Once the probe is thawed, use with two months.

9.3 **Converter Enzyme III/Conversion Enzyme:**

Ready to use as supplied. Keep on ice while in use. Store at -20°C.

9.4 **Acetyl CoA Enzyme Mix:**

Ready to use as supplied. Keep on ice while in use. Store at -20°C.

9.5 **Substrate Mix:**

Reconstitute the Acetyl CoA Substrate Mix in 220 µL of Assay Buffer XXII/Assay Buffer. Pipette up and down to completely dissolve. Store at -20°C. Use within 2 months.

9.6 **CoA Quencher:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.7 **Quench Remover I/Quench Remover:**

Dissolve Quench Remover I/Quench Remover in 220 µL ddH_2O. Keep on ice while in use. Store at -20°C.

9.8 **Acetyl CoA Standard:**

Reconstitute the Acetyl CoA Standard in 100 µL of ddH_2O to generate a 10 mM Acetyl CoA standard stock solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For 0-500 pmol range:**

10.1.1 Prepare 0.1 mM Acetyl CoA standard by adding 5 µL of Acetyl CoA standard into 495 µL ddH_2O.

10.1.2 Prepare 0.02 mM Acetyl CoA standard by adding 100 µL of 0.1 mM Acetyl CoA standard into 400 µL ddH_2O.

10.1.3 Using 0.02 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	dH_20 (µL)	Final volume standard in well (µL)	End [Acetyl CoA] in well
1	0	150	50	0 pmol/well
2	15	135	50	100 pmol/well
3	30	120	50	200 pmol/well
4	45	105	50	300 pmol/well
5	60	90	50	400 pmol/well
6	75	75	50	500 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 **For 0-100 pmol range:**

10.2.1 Prepare 0.1 mM (100 pmol/µL) Acetyl CoA standard by adding 5 µL of Acetyl CoA standard into 495 µL ddH_2O.

10.2.2 Prepare 2 µM (2 pmol/µL) Acetyl CoA standard by adding 10 µL of 0.1 mM Acetyl CoA standard into 490 µL ddH_2O.

10.2.3 Using 2 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	dH_2O (µL)	Final volume standard in well (µL)	End [Acetyl CoA] in well
1	0	150	50	0 pmol/well
2	30	120	50	20 pmol/well
3	60	90	50	40 pmol/well
4	90	60	50	60 pmol/well
5	120	30	50	80 pmol/well
6	150	0	50	100 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest ~2X10^6 cells

11.1.2 Suspend the cell pellet in 500 µl of the Assay Buffer XXII/assay buffer on ice.

11.1.3 Homogenize using a Dounce homogenizer on ice for 10-50 passes until efficient lysis is confirmed by viewing the cells under a microscope.

11.1.4 Spin homogenate at 10,000g for 10 min at 4C.

11.1.5 Collect the supernatant on ice.

11.1.6 Perform deproteinization as described in section 11.3.

11.2 Tissue Samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 20 – 1,000 mg). Tissue should be frozen rapidly (liquid N_2 or methanol/dry ice), weighed and pulverized.

11.2.2 Add 2 µL 1N perchloric acid/mg sample. KEEP COLD!

11.2.3 Homogenize or sonicate thoroughly.

11.2.4 Spin homogenate at 10,000 x g.

11.2.5 Neutralize supernatant with 3M $KHCO_3$, adding repeated 1 µL aliquots/10 µL supernatant while vortexing until bubble evolution ceases (2 - 5 aliquots).

11.2.6 Put on ice for 5 minutes.

11.2.7 Check pH (using 1 µL) should be ~ pH 6 - 8.

11.2.8 Spin 2 minutes to pellet KOH. Collect supernatant and keep on ice. Sample is ready to be assayed – step 11.3 is unnecessary. Proceed directly with step 12.1.

11.3 **Deproteinization step:**

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.3.1 Add ice cold PCA to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE:*** *high protein concentration samples might need more PCA.*

11.3.2 Incubate on ice for 5 minutes.

11.3.3 Centrifuge samples at 13,000 *x g* for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube.

11.3.4 Precipitate excess PCA by adding ice-cold 2 M KOH that equals 34% of the supernatant to your samples (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA. There may be some gas (CO_2) evolution so vent the sample tube.

11.3.5 After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust the pH with 0.1 M KOH.

11.3.6 Centrifuge at 13,000 *x g* for 15 minutes at 4°C and collect supernatant.

11.3.7 Transfer supernatant to a clean tube, and keep on ice.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\% \text{ original concentration} = \frac{\text{Initial sample volume}}{\text{(initial sample volume + vol PCA + vol KOH)}} \times 100$$

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 10 µL samples (adjust volume to 50 µL/well with Assay Buffer XXII/Assay Buffer).
- Background sample wells: 10 µL samples (adjust volume to 50 µL/well with Assay Buffer XXII/Assay Buffer).

12.2 Add 10 µL of CoA Quencher to standard, sample and sample background wells. (Free CoA and succ-CoA in samples generates background – this step quenches free CoA).

12.3 Incubate at room temperature for 5 minutes.

12.4 Add 2 µL of Quench Remover I/Quencher Remover to standard, sample and background sample wells. Mix well.

12.5 Incubate at room temperature for 5 minutes.

12.6 **Reaction Mix:**

Prepare Reaction Mix for each reaction as per below depending on your standard curve range.

Background reaction mix should be prepared to correct for succ-CoA (or other forms) by omitting the Converter Enzyme III/conversion enzyme, in duplicate sample wells.

Component	0.1 nmol Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XXII/Assay Buffer	**40**	**41**
Substrate Mix	**2**	**2**
Converter Enzyme III/Conversion Enzyme	**1**	**0**
Enzyme Mix	**5**	**5**

PicoProbe II/PicoProbe	**2**	**2**

Component	0-100 pmol Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XXII/Assay Buffer	**41.8**	**42.8**
Substrate Mix	**2**	**2**
Converter Enzyme III/Conversion Enzyme	**1**	**0**
Enzyme Mix	**5**	**5**
PicoProbe II/PicoProbe	**0.2**	**0.2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.7 Add 50 µL of Reaction Mix to each standard and sample well.

12.8 Add 50 µL of Background Reaction Mix to background sample wells.

12.9 Incubate at 37ºC for 10 minutes.

12.10 Measure output on a microplate reader.

- Fluorometric assay: measure Ex/Em = 535/587 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Subtract the sample background from sample readings if applicable.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Acetyl CoA.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Ay = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of acetyl CoA (pmol/µL, nmol/mL or µM) in the test samples is calculated as:

$$Acetyl\ CoA = \frac{Ay}{Sv}$$

Where:

Ay = Amount of Acetyl CoA in sample well (pmol).

Sv = Sample volume (µL) added to the reaction well.

Acetyl CoA molecular weight: 809.6 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

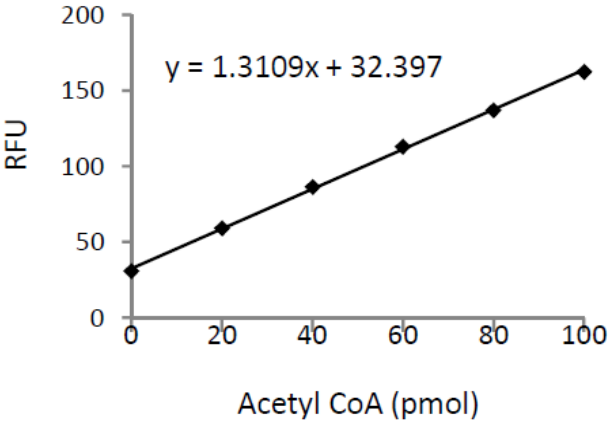


Figure 2: Typical Acetyl CoA 100 pmol standard calibration curve using fluorometric reading.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, PicoProbe II/Pico probe and prepare substrate mix (aliquot if necessary); get equipment ready
- Prepare appropriate range of standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings)
- Add 10 µL CoA Quencher to standard, sample and sample background wells.
- Incubate RT 5 mins.
- Add 2 µL Quench Remover I/Quencher Remover to standard, sample and sample background wells.
- Incubate RT 5 mins.
- Prepare Reaction Mix (Number samples + standards + 1).

Component	0.1 nmol Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XXII/Assay Buffer	**40**	**41**
Substrate Mix	**2**	**2**
Converter Enzyme III/Conversion Enzyme	**1**	**0**
Enzyme Mix	**5**	**5**
PicoProbe II/PicoProbe	**2**	**2**

Component	0-100 pmol Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XXII/Assay Buffer	**41.8**	**42.8**
Substrate Mix	**2**	**2**
Converter Enzyme III/Conversion Enzyme	**1**	**0**

Enzyme Mix	**5**	**5**
PicoProbe II/PicoProbe	**0.2**	**0.2**

- Add 50 µL Reaction Mix to each standard and sample wells.
- Add 50 µL Background Reaction Mix to each background sample wells.
- Incubate 37ºC 10 mins.
- Measure output on a microplate reader Ex/Em = 535/587 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

When you add the CoA conversion to the samples which reaction mix should you add? The 0-1 nmol mix or the 0-100 pmol mix?

Add the same range reaction mix as the standard you are making. For example, if you suspect your samples to have low Acetyl CoA, use the 0-100 pmol standards and reaction mix.

For sample preparation from cultured cells, is it absolutely necessary to follow the specific cell lysis buffer provided in your protocol? We already have a particular cell lysis protocol we follow regularly.

It is crucial to do the sample preparation exactly as recommended. As acetyl CoA is very sensitive to degradation you risk the possibility of inefficient results if you make changes to the recommended protocol.

For the deproteinization step, you recommend the use of the perchloric acid method, could this be substituted by using a 10kD MW cut-off spin column?

The deproteinization approach though not as sensitive as the sample prep, would still benefit from using PCA rather than the 10 kDa spin filters. However, you can use the spin filters if you do not have the option of using PCA.

18. INTERFERENCES

19. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)