PicoProbe™ Acetyl CoA Fluorometric Assay Kit

(Catalog #K317-100; 100 assays; Store Kit at -20° C)

I. Introduction:

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. BioVision has developed 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 to generate fluorescence (Ex/Em =535/587 nm). The assay can detect 10 to 1000 pmol of Acetyl CoA (with detection limit ~ 0.4 µM) in a variety of samples.

II. Kit Contents:

Components	K317-100	Cap Code	Part Number
Acetyl CoA Assay Buffer	25 ml	WM	K317-100-1
PicoProbe	0.2 ml	Blue	K317-100-2
Conversion Enzyme	0.1 ml	Green	K317-100-3
Acetyl CoA Enzyme Mix	0.5 ml	Purple	K317-100-4
Acetyl CoA Substrate Mix	lyophilized	Red	K317-100-5
CoA Quencher	1.0 ml	Orange	K317-100-6
Quench Remover	lyophilized	Clear	K317-100-7
Acetyl CoA Standard (1 µmol)	lyophilized	Yellow	K317-100-8

III. Storage and Handling:

Store kit at –20° C, protect from light. Warm Acetyl CoA Assay Buffer to room temperature prior to using it. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions:

PicoProbe: in DMSO, ready to use as supplied. Thaw by warming to room temperature. Mix well, store at –20° C.

Substrate Mix: Dissolve with 220 µl Assay Buffer. Pipette up and down to completely dissolve. Store at –20° C. Use within two months.

Quench Remover: Dissolve in 220 µl dH2O. Keep on ice while in use, store at -20° C.

Acetyl CoA Standard: Dissolve in 100 µl dH2O to generate 10 mM (10 nmol/µl) Acetyl CoA Standard solution. Keep cold while in use. Store at -20° C.

V. Acetyl CoA Assay Protocol:

1. Acetyl CoA Standard Curve Preparations:

0 - 1 nmol Range: Dilute the Acetyl CoA Standard 100X to 0.1 mM (100 pmol/µl) by taking 10 µl into 990 µl dH_2O. Dilute a further 5X to 0.02 mM by adding 100 µl to 400 µl dH_2O. Add 0, 10, 20, 30, 40, 50 µl into a series of wells in a 96-well plate. Adjust volume to 50 µl/well with dH2O to generate 0, 200, 400, 600, 800, 1000 pmol/well Acetyl CoA standard.

0 - 100pmol Range: Dilute the Acetyl CoA Standard 100X to 0.1 mM (100 pmol/µl) by taking 10 µl into 990 µl dH_2O. Dilute an additional 50X to 2 µM (2 pmol/µl) by taking 10 µl into 490 µl of dH_2O. Mix well. Add 0, 10, 20, 30, 40, 50 µl into a series of standards wells on a 96 well plate. Adjust volume to 50 µl/well with dH2O to generate 0, 20, 40, 60, 80, 100 pmol/well Acetyl CoA standard.

Sample Preparation: Enzymes in samples interfere with the assay. You should deproteinize your sample using a perchloric acid/KOH protocol (BioVision, Cat. #K808-200). Tissue samples (20 - 1000 mg) should be frozen rapidly (liquid N_2 or methanol/dry ice), weighed and pulverized.

Add 2 µl 1N perchloric acid/mg sample. KEEP COLD! Homogenize or sonicate thoroughly. Spin homogenate at 10,000 x g. Neutralize supernatant with 3 M $KHCO_3$, adding repeated 1 µl aliquots/10 µl supernatant while vortexing until bubble evolution ceases (2 - 5 aliquots). Put on ice for 5 min. Check pH (using 1 µl) should be ~ pH 6 - 8. Spin 2 min to pellet $KClO_4$. Add 10 µl of sample into duplicate wells (Sample and Background) of a 96-well plate; bring volume to 50 µl with Assay Buffer.

2. Free CoASH, malonyl CoA, and succ-CoA in samples generate background. In order to correct for this background, add 10 µl of CoA Quencher to each Standard, Sample and background sample to quench free CoA. Incubate for 5 min at room temp. Then add 2 µl of Quench Remover, mix and incubate 5 min. In addition, run background control for each sample to correct for succ-CoA 9or some other forms by omitting the Conversion Enzyme.
3. **CoA Conversion:** Make up 50 µl of reaction mix for each well to be tested (Standard, Sample and Background):

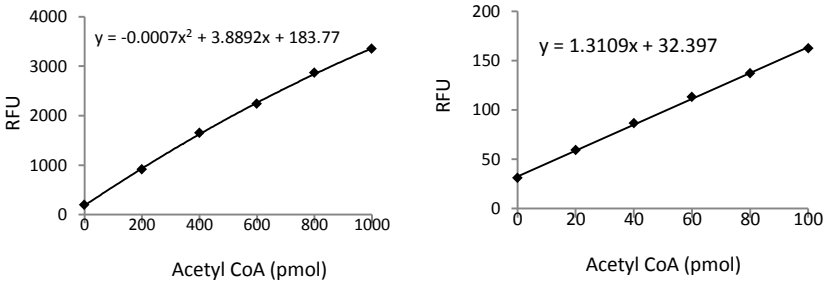
	0 – 1 nmol	Bkgd	0-100 pmol	Bkgd
Buffer:	40 µl	41 µl	41.8 µl	42.8 µl
Substrate Mix:	2 µl	2 µl	2 µl	2 µl
Conversion Enzyme:	1 µl	----	1 µl	----
Enzyme Mix:	5 µl	5 µl	5 µl	5 µl
PicoProbe:	2 µl	2 µl	0.2 µl	0.2 µl

4. **Incubate** for 10 min at 37° C.
5. Measure fluorescence using Ex/Em = 535/587 nm with a plate reader.
6. **Calculation:** Correct background by subtracting the value of the 0 Acetyl CoA Standard from all readings (Note: The background reading can be significant and must be subtracted from sample readings). Determine Background values for each sample tested and correct Acetyl CoA values for this background. Plot the Standard Curve. Apply the sample readings to the Standard Curve to get the Acetyl CoA amount in the sample wells.

   The Acetyl CoA concentrations in the test samples:

   C = Ay/Sv (pmol/µl; or nmol/ml; or µM)

   Where: **Ay** is the amount of Acetyl CoA (pmol) in your sample from the Standard Curve.
   Sv is the sample volume (µl) added to the sample well.
   Acetyl CoA molecular weight: 809.6



Standard curves were generated following this kit protocol.

RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Cell Proliferation & Senescence Kits
Glucose and Sucrose Assay Kit	Cholesterol, LDL/HDL Assay Kits
Glutathione Assay Kit	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit	Lactate Assay Kits
Pyruvate Assay Kit	Fatty Acid Assay Kit

Note: White plates enhance the sensitivity of fluorescent asays and are highly recommended

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates or white plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		