DATA SHEET

For research use only

PicoProbe™ Acetyl CoA 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=535/Em=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 (10 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 10,000Xg. Neutralize supernate with 3M $KHCO_3$, adding repeated 1μl aliquots/10 μl supernate while vortexing. Add until bubble evolution ceases (2-5 aliquots). Put on ice 5 minutes. Check pH (using 1 μl) should be ~6-8. Spin 2 minutes to pellet $KClO_4$. Add 10 μl sample into duplicate wells (Sample and Background) of a 96-well plate; bring volume to 50 μl with Assay Buffer.

2. Free CoASH and succ-CoA in samples generate background. In order to correct for this background, add 10 μl of CoASH Quencher to each background sample to quench free CoA. Incubate for 5 minutes at room temp. Then add 2 μl of Quench Remover, mix and incubate 5 minutes.

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 minutes at 37°C.

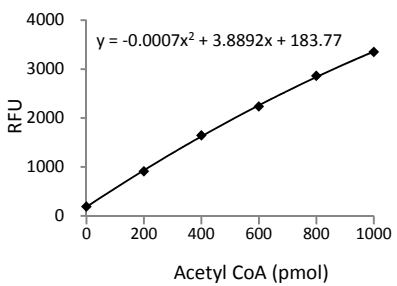
5. Measure fluorescence using Ex/Em=535/589nm with a plate reader.

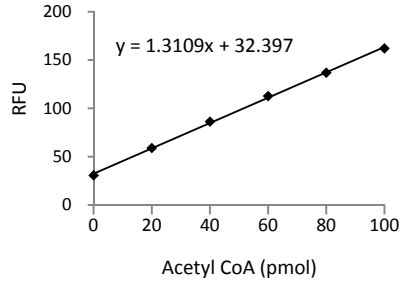
6. **Calculation:** Correct background by subtracting the value of the 0 Acetyl CoA control from all readings (Note: The background reading can be significant and must be subtracted from sample readings). Determine Background values for each sample tested and correct Acetyl CoA values for this background. Plot the standard curve. Apply the sample readings to the standard curve to get 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.

VI. 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

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