Product Information

Acetyl-CoA Fluorometric Assay Kit

I. Kit Contents:

Components	K2028-100 100 assays	Cap Color	Part Number
Acetyl CoA Assay Buffer	25 ml	WM	K2028-C-1
PicoProbe	0.2 ml	Blue	K2028-C-2
Conversion Enzyme	0.1 ml	Green	K2028-C-3
Acetyl CoA Enzyme Mix	0.5 ml	Purple	K2028-C-4
Acetyl CoA Substrate Mix	lyophilized	Red	K2028-C-5
CoA Quencher	1.0 ml	Orange	K2028-C-6
Quench Remover	lyophilized	Clear	K2028-C-7
Acetyl CoA Standard (1 µmol)	lyophilized	Yellow	K2028-C-8

II. Introduction:

Acetyl CoA is a critical molecule in metabolism. Acetyl CoA is an important molecule in the citric acid cycle to be oxidized for energy production. Also, Acetyl CoA is a key molecule in the biogenic synthesis of acetylcholine, melatonin, heme, sesquiterpenes, polyenes and long-chain fatty acids, precursors to cholesterol and other sterols, flavenoids and other polyketides. Acetyl CoA is also the source of the acetyl group used in histone acetylation.

The Acetyl-CoA Fluorometric Assay Kit provides a convenient and highly sensitive way for the quantification of Acetyl CoA level in variety of biological samples. In the assay, free CoA is quenched and then Acetyl CoA is converted to CoA. The CoA is reacted to produce NADH which interacts with PicoProbe to produce fluorescence (Ex = 535/Em = 587 nm). The assay can quantify 10 to 1000 pmol of Acetyl CoA in a variety of samples with detection limit ~0.4 µM.

III. Storage and Handling:

Store kit at -20℃, 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:

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

Quench Remover: Dissolve in 220 µl dH_2O. Keep on ice while in use, store at -20℃.

Acetyl CoA Standard: Dissolve in 100 µl dH_2O to generate 10 mM (10 nmol/µl) Acetyl CoA.

Standard solution. Keep cold while in use. Store at -20℃.

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 dH_2O 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 dH_2O 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. Tissue samples (20 - 1000 mg) should be frozen rapidly (liquid N2 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 KHCO3, 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 KClO4. 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 and succ-CoA in samples generate background. In order to correct for this background, add 10 µl of CoASH Quencher to each Standard, Sample and background sampleto 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	2 µl	2 µl

4. Incubate for 10 min at 37℃.

5. Measure fluorescence using Ex/Em = 535/589 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 si gnificant 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

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument

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

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