MA-0127

Acetyl CoA Assay

(100 wells, Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Acetyl CoA is a key molecule in cellular metabolism and energy production. A central hub, connecting different metabolic pathways within the cell, acetyl CoA plays a pivotal role in the citric acid cycle, resulting in the production of energy-rich molecules such as ATP and is a precursor for the synthesis of fatty acids, cholesterol, and other important cellular components. Its versatility and significance make acetyl CoA a fundamental molecule for sustaining cellular processes and maintaining overall metabolic homeostasis. Several enzyme deficiencies have a detrimental effect on Acetyl CoA metabolism: Among them are Pyruvate DH deficiency, maple syrup urine disease, ketothiolase deficiency, HMG-CoA lyase deficiency and multiple acyl-CoA dehydrogenase deficiency. AkrivisBio's Acetyl CoA Assay is a simple, sensitive method of measuring amounts of Acetyl CoA in a variety of samples with a useful detection range of 0 – 400 picomoles.

Assay Principle:

1 – Acetyl CoA present in samples is hydrolyzed to Coenzyme A and acetate
2 – In the presence of NAD and ketoglutarate, ketoglutarate dehydrogenase converts the CoA to succinyl CoA forming NADH in the process
3 – The NADH is utilized by diaphorase to reduce resazurin to resorufin with intense fluorescence

Kit Components:

Assay Buffer	25 ml	WM	MA-0127-A
Resazurin	0.2 ml	Blue	MA-0127-B
Phosphotransacetylase	0.1 ml	Green	MA-0127-C
Ketoglutarate DH/Diaphorase	0.5 ml	Purple	MA-0127-D
Ketoglutarate	lyophilized	Red	MA-0127-E
Quenching Agent	1.0 ml	Orange	MA-0127-F
Quench Neutralizer	lyophilized	Clear	MA-0127-G
Acetyl CoA Standard	lyophilized	Yellow	MA-0127-H

Storage and Handling: Store kit at -20°C. Centrifuge all vials briefly before opening.
Assay Buffer: Warm Assay Buffer to room temperature prior to use. Store at 4°C
Resazurin; Quenching Agent:: Ready to use as supplied. Thaw by warming to room temperature. Store at 4°C.
Ketoglutarate: Dissolve in 220 µl Assay Buffer. Store at –20°C.
Quench Neutralizer: Dissolve in 220 µl DI water. Keep on ice while in use. Store at -20°C.
Acetyl CoA Standard: Dissolve in 100 µl DI water, gives a 10 mM solution. Keep on ice while in use. Store at -20°C.

Assay Protocol:

1. Standard Curve:

0 – 500 pmol Range: Dilute the Acetyl CoA Standard 100X, transferring 10 µl to 990 µl DI water. Dilute further, transferring 20 µl to 180 µl H_2O. The final working solution is 10 µM. Transfer 0 – 10 – 20 – 30 – 40 – 50 µl into a series of wells in a 96-well plate. Adjust all well volumes to 50 µl with DI water giving 0, 100, 200, 300, 400, 500 pmol/well Acetyl CoA standard.

0 – 100 pmol Range: Dilute the Acetyl CoA Standard 100X, transferring 10 µl to 990 µl DI water. Dilute further, transferring 10 µl to 490 µl of DI water. Mix well. Transfer 0 – 10 – 20 – 30 – 40 – 50 µl into a series of wells in a 96-well plate. Adjust all well volumes to 50 µl with DI water giving 0, 20, 40, 60, 80, 100 pmol/well Acetyl CoA standard.

2. Sample Preparation: Enzymes in samples rapidly degrade acetyl CoA interfering with the assay. Deproteinize samples using PI-0102, PI-0103 or equivalent. Tissue samples (20 - 1000 mg) should be frozen rapidly (liquid N_2 or methanol/dry ice), weighed and pulverized. Add 2 µl of 1 N perchloric acid/mg sample. Keep cold, disrupt samples by homogenization or sonication. Centrifuge at 16,000 X g. Neutralize supernatant with 3 M $KHCO_3$, adding 1 µl aliquots/10 µl supernatant while vortexing until bubble evolution ceases (2 - 5 aliquots). Place on ice for 5 min. Check pH (using 1 µl) should be ~ pH 6 - 8. Spin at 16,000 X g for 2 min to pellet $KClO_4$. Transfer 10 µl of sample to paired wells in a 96-well plate; adjust all well volumes to 50 µl with Assay Buffer.

Free CoASH, malonyl CoA, and succ-CoA in samples generate background. To correct for this background, add 10 µl of Quenching Agent to all standards, samples and background controls to quench free CoA. Incubate for 5 min at room temp then add 2 µl of Quench Neutralizer, mix and incubate 5 min. In addition, run a background control for each sample to correct for succ-CoA or other CoA esters by omitting the Conversion Enzyme.

3. Initiate Reaction: Each reaction requires 50 µl of reaction mix. Prepare sufficient reaction mix for the total number of wells to be run:

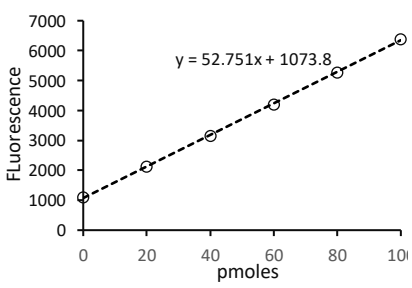
	0 – 500 pmol	Bkgd	0-100 pmol	Bkgd
Assay Buffer:	40 µl	41 µl	41.8 µl	42.8 µl
Ketoglutarate:	2 µl	2 µl	2 µl	2 µl
Phosphotransacetylase:	1 µl	----	1 µl	----
Ketoglutarate DH/Diaphorase:	5 µl	5 µl	5 µl	5 µl
Resazurin Solution:	2 µl	2 µl	0.2 µl	0.2 µl

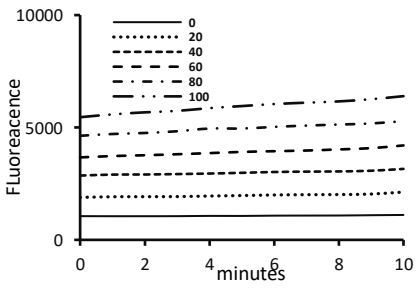
Incubate for 10 min at 37°C.

4. Measurement: Monitor fluorescence using Excitation 535 nm, Emission 587 nm with a plate reader. There is a very slow linear autocatalytic reaction which occurs proportional to the amount of acetyl CoA present. To correct for this, determine the rate of drift between 10 – 15 minutes after initiation of the reaction and extrapolate that rate back to 0 time (time of addition of reaction mix)

5. Typical results:

pmole Standard	Standard Raw Values (Fluorescence)	Background Corrected Values (Fluorescence)
0	1108.52	0
20	2127.823	1019.303
40	3156.68	2048.16
60	4201.509	3092.989
80	5281.105	4172.585
100	6392.715	5284.195





6. Calculation: Subtract 0 standard from all readings. Background can be significant and must be subtracted from sample readings. Determine Background values for each sample tested and subtract from raw acetyl CoA readings to get corrected Acetyl CoA fluorescence. Plot the Standard Curve and determine the slope of the standard curve. Apply the slope of the standard curve to the corrected sample readings to get the pmoles of Acetyl CoA in the sample wells. Determine acetyl CoA in original sample:

A. pmole of acetyl CoA in well / µl added to well = pmoles/µl of sample
B. pmoles/µl of sample X total volume of sample = total acetyl CoA in sample
C. total acetyl CoA in sample / mg tissue (or # of cells) = pmoles acetyl CoA / mg (# cells, etc.)

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