MA-0130

Acetate Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Background Information:

Acetate, derived from several metabolic pathways, plays a central role in energy metabolism. As an intermediate, acetate is a substrate in the TCA cycle, the central hub of cellular respiration. Once generated, it is oxidized in the cycle, resulting in ATP formation. Acetate also serves as a precursor for lipid synthesis, contributing to the formation of fatty acids and cholesterol. Moreover, acetate is a signaling molecule, regulating gene expression and cellular processes. Acetate's metabolic importance extends beyond energy production, encompassing various biochemical pathways critical for cell function and homeostasis. AkrivisBio's Acetate Assay is a simple sensitive means of measuring acetate concentrations in a variety of biological samples with a sensitivity below 1 nanomole per well.

Assay Principle

1 – Acetate kinase convert acetate to acetyl phosphate and ATP to ADP
2 – The ADP is used to phosphorylate glucose to glucose-6-phosphate
3 – glucose-6-phosphate converts NAD to NADH
4 – NADH reduced a nearly colorless tetrazolium to a highly colored formazan (λ_{max} = 450 nm)

Assay Components:

Assay Buffer	25 ml	WM	MA-0130A
Acetate Kinase/Hexokinase	Lyoph	Green	MA-0130B
ATP	Lyoph	Orange	MA-0130C
Glucose	Lyoph	Blue	MA-0130D
NAD/Tetrazolium Mix	Lyoph	Red	MA-0130E
Acetate Standard	50 µl	Yellow	MA-0130F

Storage and Handling:

Store kit at -20°C prior to use. Warm Assay components to room temperature before use. Centrifuge all small vials for several seconds prior to opening.
Assay Buffer: ready to use as supplied once thawed. Store at 4°C
Acetate Kinase/Hexokinase: Reconstitute with 220 µl Assay Buffer. If the assay is to be used over a period of time,aliquot the enzymes into convenient portions and store at -20°C, Thaw only what's necessary for a day's tests. Avoid repeated freeze- thaw cycles. Keep on ice while in use.
ATP; NAD/Tetrazolium Mix: Reconstitute each with 220 µl DI H_2O. Keep on ice while in use. Store at -20°C
Glucose: Dissolve with 220 µl Assay Buffer. Store at -20°C.
Acetate Standard: Ready to use as supplied. Standard is 40 mM. Keep on ice while in use. Store at -20°C.

Assay Protocol:

Note: Acetate in the air interferes with the assay. Ensure that no open acetic acid containers are in the laboratory where the assay is used.

1. Standard Curve: Dilute 40 mM Acetate Standard to 0.4 mM by adding 10 µl to 990 µl DI H_2O. Add 0 – 5 – 10 – 15 – 20 – 25 µl of 0.4 mM Acetate Standard to a series of wells in a 96 well plate, giving 0 – 2 – 4 – 6 – 8 and 10 nmol/well of Acetate Standard. Adjust all wells to 50 µl with Assay Buffer.

2. Samples: Homogenize tissue (10 mg) or cells (1 x 10^6) thoroughly with 100 µl ice cold Assay Buffer. Centrifuge at 16,000 X g for 5 min. Transfer the clear supernatant to a fresh tube. Add up to 50 µl of each sample to wells of a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer.

Notes:
- All samples must give readings within the range of the standard curve. If any samples exceed this range, dilute and rerun.
- ADP & NADH in samples generate background. Typically, acetate concentrations are much higher so background is usually insignificant. If you suspect that ADP or NADH contribute significantly to the signal, run samples in duplicate with the paired sample used as a background control well.
- Enzymes (rarely) interfere with the assay and can be removed by using a 10 kDa centrifugal filter.

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

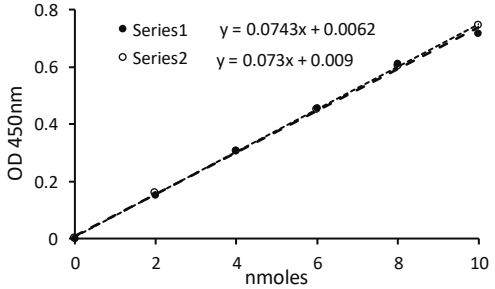
	Reaction Mix	Background Control Mix
Assay Buffer	42 µl	44 µl
Acetate Kinase/Hexokinase	2 µl	----
ATP	2 µl	2 µl
Glucose	2 µl	2 µl
NAD/Tetrazolium Mix	2 µl	2 µl

Add 50 µl of Reaction Mix to wells containing Standards or test samples. Add 50 µl of Background Control Mix to paired background control wells.

4. Measurement: Monitor the absorbance at room temperature at 450 nm.

5. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.2031	0.2014	0	0
2	0.3555	0.3620	0.1524	0.1606
4	0.5099	0.5065	0.3068	0.3051
6	0.6596	0.6537	0.4565	0.4523
8	0.8144	0.8034	0.6113	0.6020
10	0.9195	0.9473	0.7164	0.7459



6. Calculation: Subtract 0 Standard reading from all standards readings. Plot the Acetate Standard Curve for the background corrected values obtained. Determine the slope of the standard curve as this will define the assay signal (OD/nmole). Subtract background control well values from paired unknowns. Divide the corrected values by the slope of the standard curve to obtain nmoles of acetate in the wells. Convert amount in sample wells to amount in original samples as follows:

A. Nmoles acetate in well / µl sample added to well = nmole acetate per µl sample
B. Nmole acetate per µl sample X total vol of sample = total nmoles acetate in sample
C. Total nmoles acetate in sample / mg tissue (or # of cells, or volume of fluid sample) = nmoles acetate / mg (or # or volume)
D. If sample was diluted due to high signal, multiply by dilution factor.

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