3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Acetate Colorimetric Assay Kit

Catalog Number **MAK086**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Acetate, the ionized form of acetic acid, is a critical metabolite in fatty acid and carbohydrate metabolism. The majority of acetate is utilized as the conjugate to coenzyme A (CoA), acetyl-CoA. Acetyl-CoA is a precursor to fatty acid and cholesterol synthesis, and an important starting compound for the citric acid cycle. Acetyl-CoA also serves as a precursor for the synthesis of acetylcholine and as the donor for acetyl groups for post-translational acetylation reactions of histone and non-histone proteins. Acetate is generated from the metabolism of carbohydrates, amino acids, fatty acids, and ethanol.

The Acetate Assay kit provides a simple and direct procedure for measuring acetate in a variety of samples such as serum, tissue, cells, and food. Acetate concentration is determined by a coupled enzyme assay, which results in a colorimetric (450 nm) product proportional to the acetate present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Acetate Assay Buffer Catalog Number MAK086A	27 mL
Acetate Enzyme Mix Catalog Number MAK086B	1 vl
ATP Catalog Number MAK086C	1 vl
Acetate Substrate Mix Catalog Number MAK086D	1 vl
Probe Catalog Number MAK086E	1 vl
Acetate Standard, 10 µmole Catalog Number MAK086F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Acetate Assay Buffer – Allow buffer to come to room temperature before use.

Acetate Enzyme Mix and Acetate Substrate Mix – Reconstitute each with 220 µL of Acetate Assay Buffer. Separately, mix well by pipetting (don't vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

ATP and Probe – Reconstitute each with 220 µL of water. Separately, mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Acetate Standard – Reconstitute with 100 µL of water to generate a 100 mM Acetate stock solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Acetate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Acetate stock solution with 990 µL of water to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Acetate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µL of ice-cold Acetate Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum and other liquid samples can be directly added to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 µL with Acetate Assay Buffer.

Notes: ADP and NADH in the samples will generate a background signal. To remove the effect of ATP and NADH background, a sample blank may be set up for each sample by omitting the Acetate Enzyme Mix.

Samples may be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction. This step may be necessary if enzymes present in the samples generate interference.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the apppropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Acetate Assay Buffer	42 µL	44 µL
Acetate Enzyme Mix	2 µL	–
ATP	2 µL	2 µL
Acetate Substrate Mix	2 µL	2 µL
Probe	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 40 minutes at room temperature. Cover the plate and protect from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) Acetate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate acetate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the value from the sample blank reading to obtain the corrected measurement. Using the corrected measurement, the amount of acetate present in the samples may be determined from the standard curve.

Concentration of Acetate

$$S_a/S_v = C$$

S_a = Amount of acetate in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added to reaction well

C = Concentration of acetate in sample

Acetic Acid molecular weight: 59.05 g/mole

Sample Calculation

Amount of acetate (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of acetate in sample

$$5.84 \text{ nmole}/50 \text{ μL} = 0.1168 \text{ nmole}/\text{μL}$$

$$0.1168 \text{ nmole}/\text{μL} \times 59.05 \text{ ng/nmole} = 6.90 \text{ ng}/\text{μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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