EnzyChrom™ Acetate Assay Kit (EOAC-100)

Quantitative Colorimetric/Fluorimetric Acetate Determination

DESCRIPTION

ACETATE is a common anion and fundamental to all forms of life. When bound to coenzyme A, it is central to the metabolism of carbohydrates and fats. Its acid form, acetic acid, is produced and excreted by acetic acid bacteria, such as *Acetobacter genus* and *Clostridium acetobutylicum*, which are found universally in foodstuffs, water, and soil. Acetic acid is also a component of the vaginal lubrication of humans and other primates, where it appears to serve as a mild antibacterial agent. Acetic acid is the main component of vinegar, and extensively used in food, dyes, paints, glue and synthetic fibres.

BioAssay Systems' assay uses enzyme-coupled reactions to form a colored, fluorescent product. The color absorbance at 570nm or fluorescence intensity at 530nm/585nm is directly proportional to the acetate concentration in the sample.

KEY FEATURES

Use as little as 10 µL samples. Detection range: 0.20 to 20 mM acetate for colorimetric assays and 0.13 to 2 mM for fluorimetric assays.

APPLICATIONS:

Direct Assays: acetate in biological samples such as serum/plasma, in food, agriculture and environmental samples.
Drug Discovery/Pharmacology: effects of drugs on acetate metabolism.

KIT CONTENTS

Assay Buffer:	25 mL	**Enzyme A:**	Dried
Developer:	1 mL	**Enzyme B:**	Dried
Dye Reagent:	120 µL	**Standard:**	1 mL
ATP:	120 µL		

Kit storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

FLUORIMETRIC PROCEDURE

Sample treatment: serum and plasma samples can be assayed directly. Acetic acid containing samples such as vinegars should be diluted in the Assay Buffer prior to assay. Samples should be clear, and free of precipitate or particles. If present, precipitate or particles should be removed by filtration or centrifugation.

1. Equilibrate all components to room temperature. Briefly centrifuge tubes. Reconstitute Enzyme A with 600 µL Developer and Enzyme B with 120 µL Assay Buffer. Make sure both enzymes are fully dissolved before proceeding with the assay. During the experiment, keep Enzymes in a refrigerator or on ice. Reconstituted Enzyme A and Enzyme B are stable for four weeks if stored at -20 ℃.

2. *Standards and samples*: prepare 400 µL 2 mM Standard by mixing 4 µL 200 mM standard with 396 µL dH_2O. Dilute standard in dH_2O as follows.

No	2 mM STD + H_2O	Vol (µL)	Acetate (mM)
1	100 µL + 0 µL	100	2.0
2	75 µL + 25 µL	100	1.5
3	50 µL + 50 µL	100	1.0
4	25 µL + 75 µL	100	0.5
5	0 µL +100 µL	100	0

Transfer 10 µL standards and 10 µL samples into separate wells of a black flat-bottom 96-well plate.

3. *Reaction*. Prepare Working Reagent, for each reaction well, by mixing 90 µL Assay Buffer, 5 µL Enzyme A, 1 µL Enzyme B, 1 µL Dye Reagent and 1 µL ATP. *Note: the Working Reagent should be prepared freshly and used within 20 min.*

Transfer 90 µL Working Reagent to each well. Mix immediately and incubate for 30 min at room temperature. Read fluorescence intensity $\lambda_{ex}/\lambda_{em}$ = 530/585nm

COLORIMETRIC PROCEDURE

For colorimetric assays, the detection range is 0 to 20 mM acetate. Prepare 0, 4, 8, 12, 16 and 20 mM acetate standards in dH_2O. Perform the assay the same as for Fluorimetric Procedure, but use a clear flat bottom 96-well plate and read OD 570nm (550-585nm).

Notes: If the calculated acetate concentration of a sample is higher than 2 mM in fluorimetric assay or 20 mM in colorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor *n*.

CALCULATION

Subtract the water blank (Std #5) value from all the standard and sample values. Plot the ΔF or ΔOD of the standards against the standard concentrations. Determine the acetate concentration of samples from the standard curve.

Conversions: 1 mM acetate equals 5.9 mg/dL, 0.0059% or 59 ppm.

GENERAL CONSIDERATIONS

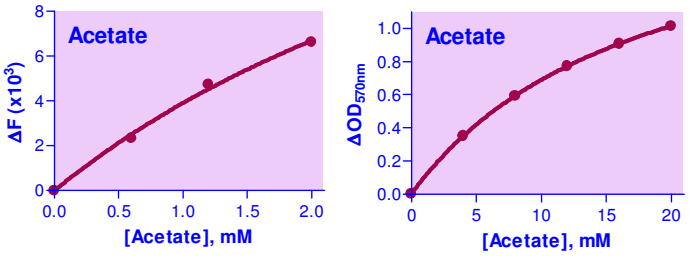
1. SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.

2. This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, optical density plate reader; black 96-well plates and fluorescence plate reader.

Acetate Standard Curves



96-well fluorimetric assay ***96-well colorimetric assay***

LITERATURE

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3. Rocchiccioli F. et al (1989). Capillary gas-liquid chromatographic/ mass spectrometric measurement of plasma acetate content and (2-13C) acetate enrichment. Biomed Environ Mass Spectrom. 18:816-9.

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