Technical Bulletin

Acetate Assay Kit

Catalogue number MAK474

Product 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 food, 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 is extensively used in food, dyes, paints, glue, and synthetic fibers.

The Acetate Assay Kit uses enzyme-coupled reactions to form a colored, fluorescent product. The color absorbance at 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/λ_{Em} = 585 nm is directly proportional to the acetate concentration in the sample

The linear detection range of the kit is 0.20 - 20 mM acetate for the colorimetric assay and 0.13 - 2 mM for the fluorometric assay. The kit is suitable for acetate determination in serum, plasma, food, agriculture, and environmental samples, as well as for studying the effects of drugs on acetate metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 25 mL
  Catalogue Number MAK474A
- Developer 1 mL
  Catalogue Number MAK474B
- Enzyme A 1 vial
  Catalogue Number MAK474C
- Enzyme B 1 vial
  Catalogue Number MAK474D
- Dye Reagent 120 µL
  Catalogue Number MAK474E
- ATP 120 µL
  Catalogue Number MAK474F
- Standard (200 mM) 1 mL
  Catalogue Number MAK474G

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or otheruses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20°C

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Enzyme A: Reconstitute vial with 600 µL of Developer. Enzyme should be fully dissolved before proceeding with the assay and should be kept on ice during the assay. Reconstituted Enzyme A is stable for four weeks if stored at -20 °C.

Enzyme B: Reconstitute the vial with 120 µL of Assay Buffer. Enzyme should be fully dissolved before proceeding with the assay and should be kept on ice during the assay. Reconstituted Enzyme B is stable for four weeks if stored at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Note: SH-containing reagents (for example, β-mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.

Serum and plasma samples can be assayed directly.

Acetic acid-containing samples such as vinegars should be diluted in 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.

Transfer 10 µL of Sample to wells of a 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare a 2 mM Acetate Standard by mixing 4 µL of 200 mM Standard with 396 µL of purified water. Prepare standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Fluorometric Acetate Standards

Well	2 mM Acetate Standard	Purified Water	Acetate (mM)
1	100 µL	0 µL	2.0
2	75 µL	25 µL	1.5
3	50 µL	50 µL	1.0
4	25 µL	75 µL	0.5
5	0 µL	100 µL	0

2. Mix well and transfer 10 µL of each Standard into separate wells of a black 96-well plate.

Colorimetric Standard Curve Preparation

1. Prepare a 20 mM Acetate Standard by mixing 40 µL of 200 mM Standard with 360 µL of purified water. Prepare standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of Colorimetric Acetate Standards

Well	20 mM Acetate Standard	Purified Water	Acetate (mM)
1	100 µL	0 µL	20
2	75 µL	25 µL	15
3	50 µL	50 µL	10
4	25 µL	75 µL	5
5	0 µL	100 µL	0

2. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

Working Reagent Preparation

Note**:** This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Mix enough reagent for the number of assays to be performed. For each well, prepare 98 µL of Working Reagent according to Table 3. Working Reagent should be prepared fresh and used within 20 minutes.

Table 3.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	90 µL
Enzyme A	5 µL
Enzyme B	1 µL
Dye Reagent	1 µL
ATP	1 µL

2. Transfer 90 µL of Working Reagent into each Sample and Standard well. Tap plate to mix.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Read optical density (OD) at 570 nm for colorimetric assay or fluorescence intensity (F) at λ_{Ex} = 530 nm/λ_{Em} = 585 nm for fluorometric assay.

Results

1. Calculate ΔOD or ΔF by subtracting the reading (OD or fluorescence intensity F) of Standard#5 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against standard concentrations and determine the slope of the standard curve.
3. Calculate the acetate concentration of Sample.

Acetate (mM) =

$$\frac{R_{SAMPLE}-R_{BLANK}}{\text{Slope } (mM^{-1})} \times DF$$

where:

R_{Sample} = OD or fluorescence intensity (F) reading of Sample

R_{Blank} = OD or fluorescence intensity (F) reading of Standard #5 (Blank)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated acetate concentration of a sample is higher than 2 mM for the fluorometric assay or 20 mM for the colorimetric assay, dilute Sample in purified water and repeat the assay. Multiply result by the dilution factor.

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

Figure 1.
Typical Fluorometric Acetate Standard Curve

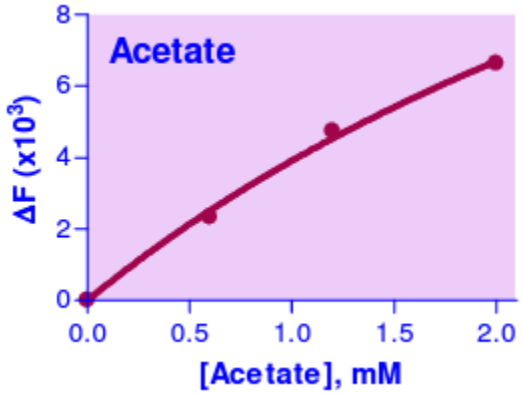
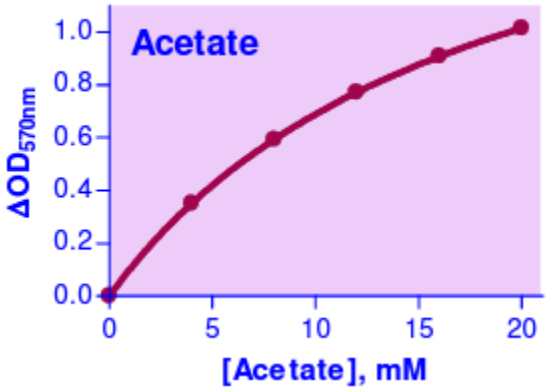


Figure 2.
Typical Colorimetric Acetate Standard Curve



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