Acetate Assay Kit (Colorimetric)

Acetate Assay Kit (Colorimetric) is a detection kit for the quantification of Acetate in serum, plasma and food.

Catalog number: ARG82126

Package: 100 tests

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INTRODUCTION

An acetate is a salt formed by the combination of acetic acid with a base (e.g. alkaline, earthy, metallic, nonmetallic or radical base). "Acetate" also describes the conjugate base or ion (specifically, the negatively charged ion called an anion) typically found in aqueous solution and written with the chemical formula $C_2H_3O_2^-$. The neutral molecules formed by the combination of the acetate ion and a positive ion (called a cation) are also commonly called "acetates" (hence, acetate of lead, acetate of aluminum, etc.). The simplest of these is hydrogen acetate (called acetic acid) with corresponding salts, esters, and the polyatomic anion $CH_3CO_2^-$, or CH_3COO^-.

Most of the approximately 5 billion kilograms of acetic acid produced annually in industry are used in the production of acetates, which usually take the form of polymers. In nature, acetate is the most common building block for biosynthesis. For example, the fatty acids are produced by connecting the two carbon atoms from acetate to a growing fatty acid. [Provide by Wikipedia: Acetate]

PRINCIPLE OF THE ASSAY

This Acetate Assay Kit (Colorimetric) is a simple assay that measures the amount of Acetate present in serum, plasma and food. This assay uses enzyme-coupled reactions to form a colored, fluorescent product. The color absorbance at O.D. 570 nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to the acetate concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	25 mL	-20°C
Developer	1 mL	-20°C
Dye Reagent	120 µL	-20°C
ATP	120 µL	-20°C
Enzyme A (lyophilized)	1 vial	-20°C
Enzyme B (lyophilized)	1 vial	-20°C
Standard	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well plate
- Deionized or distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- SH-containing reagents (E.g., β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C.

Acetic acid containing samples: 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.

REAGENT PREPARATION

Reconstitute Enzyme A: add 600 µL of Developer to the Enzyme A tube. Make sure enzyme is fully dissolved before assay. During the experiment, keep Enzymes in a refrigerator or on ice. Reconstituted Enzyme A is stable for four weeks if stored at-20°C.

Reconstitute Enzyme B: add 120 µL of Assay Buffer to the Enzyme B tube. Make sure enzyme is fully dissolved before assay. During the experiment, keep Enzymes in a refrigerator or on ice. Reconstituted Enzyme B is stable for four weeks if stored at-20°C.

Standards: mix 4 µL of Standard with 396 µL of distilled water to the final concentration 2 mM. Dilute Standard in distilled water as follow.

Standard tube	Acetate (mM)	Distilled water (µL)	Standard stock, 2 mM (µL)
S1	2.0	0	100
S2	1.5	25	75
S3	1.0	50	50
S4	0.5	75	25
S0	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge all tubes prior to use.

FLUORIMETRIC PROCEDURE

1. Add 10 µL standards and 10 µL samples into separate wells of a black flat-bottom 96-well plate.
2. Prepare **Working Reagent**, for each reaction well, by mixing **90 µL** of **Assay Buffer**, **5 µL** of **Enzyme A**, **1 µL** of **Enzyme B**, **1 µL** of **Dye Reagent** and **1 µL** of **ATP. Note:** the Working Reagent should be prepared freshly and used within 20 minutes.
3. Add **90 µL** of **Working Reagent** to each well. Mix immediately. Incubate for **30 minutes** at **room temperature**.
4. Read the fluorescence intensity at **λex/em = 530/585 nm.**

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 distilled water. Perform the assay the same as for **FLUORIMETRIC PROCEDURE**, but use a clear flat bottom 96-well plate and read the absorbance at **O.D. 570 nm (550-585 nm)**.

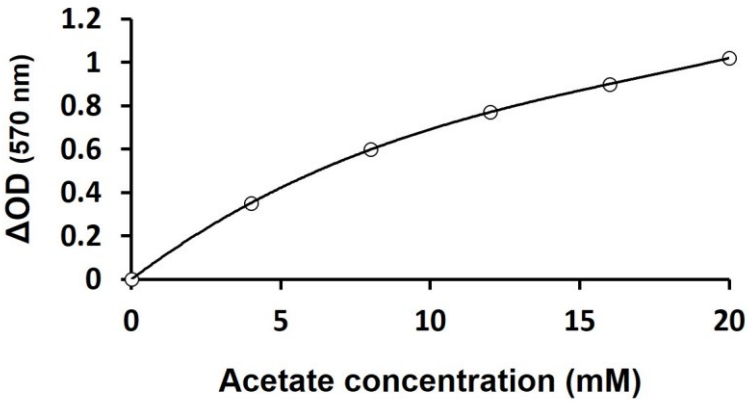
Note: If the calculated acetate concentration of a sample is higher than 2 mM in fluorimetric assay or 20 mM in colorimetric assay, dilute sample in distilled water and repeat the assay. Multiply result by the dilution factor n.

CALCULATION OF RESULTS

1. Subtract the distilled water blank (S0) 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.
2. Conversions: 1 mM acetate equals 5.9 mg/dL, 0.0059% or 59 ppm.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Acetate Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.13 mM