

## 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  $\mu$ L 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

<b>Assay Buffer:</b>	25 mL	<b>Enzyme A:</b>	Dried
<b>Developer:</b>	1 mL	<b>Enzyme B:</b>	Dried
<b>Dye Reagent:</b>	120 $\mu$ L	<b>Standard:</b>	1 mL
<b>ATP:</b>	120 $\mu$ L		

**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  $\mu$ L Developer and Enzyme B with 120  $\mu$ L 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 °C.

2. **Standards and samples:** prepare 400  $\mu$ L 2 mM Standard by mixing 4  $\mu$ L 200 mM standard with 396  $\mu$ L dH<sub>2</sub>O. Dilute standard in dH<sub>2</sub>O as follows.

No	2 mM STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Acetate (mM)
1	100 $\mu$ L + 0 $\mu$ L	100	2.0
2	75 $\mu$ L + 25 $\mu$ L	100	1.5
3	50 $\mu$ L + 50 $\mu$ L	100	1.0
4	25 $\mu$ L + 75 $\mu$ L	100	0.5
5	0 $\mu$ L + 100 $\mu$ L	100	0

Transfer 10  $\mu$ L standards and 10  $\mu$ L samples into separate wells of a black flat-bottom 96-well plate.

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

Transfer 90  $\mu$ L Working Reagent to each well. Mix immediately and incubate for 30 min at room temperature. Read fluorescence intensity  $\lambda_{ex}/\lambda_{em} = 530/585$ 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 dH<sub>2</sub>O. 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  $\Delta F$  or  $\Delta OD$  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

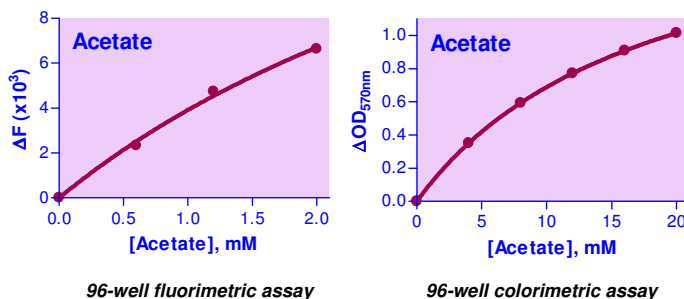
1. SH-containing reagents (e.g.  $\beta$ -mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.

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



#### PUBLICATIONS

- Cuervo-Soto, LI et al (2015). Identification of a novel carbohydrate esterase from *Bjerkandera adusta*: Structural and function predictions through bioinformatics analysis and molecular modeling. *Proteins: Structure, Function, and Bioinformatics* 83(3): 533-546.
- Inagaki, T., & Matsuura, K. (2018). Extended mutualism between termites and gut microbes: nutritional symbionts contribute to nest hygiene. *The Science of Nature*, 105(9-10), 52.
- Balmer, M. L., et al (2020). Memory cd8+ t cells balance pro- and anti-inflammatory activity by reprogramming cellular acetate handling at sites of infection. *Cell Metabolism*, 32(3), 457-467.e5.

