Acetate Colorimetric Assay Kit (#BN00882)

(Catalog #BN00882; 100 assays; Store at -20°C)

I. Introduction:

Acetate is an important component for biosynthesis. In living organisms, acetylation/deacetylation of proteins is critical for post-translational regulation of their functions. When bound to CoA, Acetyl-CoA is central to metabolism of carbohydrates and fats. It is also involved in the synthesis of biogenic compounds such as neurotransmitter acetylcholine. In Assay Genie's Acetate Assay Kit, Acetate is converted to an intermediate in the presence of Acetate Enzyme Mix & Acetate Substrate Mix. The intermediate reduces the probe to a colored product with strong absorbance at 450 nm. The Acetate Colorimetric Assay Kit is rapid, simple & sensitive. It can detect less than 20 µM Acetate in various samples.

Acetate + Acetate Enzyme Mix + Acetate Substrate Mix + ATP ⟶ Intermediate

Intermediate $\xrightarrow{\text{Probe}}$ Color detection (OD 450 nm)

II. Application:

- Measurement of Acetate in various tissues/cells
- Analysis of metabolism and cell signaling in various cells

III. Sample Type:

Serum & plasma
Animal tissues: Liver, kidney, muscle, heart etc.
Cell culture: Adherent or suspension cells
Food

IV. Kit Contents:

Components	BN00882	Cap Code	Part Number
Acetate Assay Buffer	27 ml	WM	BN00882-1
Acetate Enzyme Mix (Lyophilized)	1 vial	Green	BN00882-2
ATP (Lyophilized)	1 vial	Orange	BN00882-3
Acetate Substrate Mix (Lyophilized)	1 vial	Blue	BN00882-4
Probe (Lyophilized)	1 vial	Red	BN00882-5
Acetate Standard (Lyophilized)	1 vial	Yellow	BN00882-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **Acetate Enzyme Mix**: Reconstitute with 220 µl Acetate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze thaw. Keep on ice while in use. Stable for 2 months.
- **ATP**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Stable for 2 months.
- **Acetate Substrate Mix**: Dissolve with 220 µl Acetate Assay Buffer. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- **Probe**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Keep on ice while in use. Stable for 2 months at -20°C.
- **Acetate Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) Acetate Standard solution. Keep on ice while in use. Store at -20°C. Use within two months.

VIII. Acetate Assay Protocol:

Note: Extreme care should be taken to ensure that no acetate vapors are in the Laboratory air where this assay is to be performed. Acetate vapors in the air will be rapidly absorbed by kit components resulting in very high background making the kit unstable.

1. **Sample Preparation:** Liquid samples can be measured directly. Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 100 µl ice cold Acetate Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (50-200 µg) into a 96 well plate. Adjust final volume to 50 µl with Acetate Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
   b. ADP & NADH in samples will generate background. If your sample contains ADP or NADH, prepare a parallel sample well as the background control to subtract interference from ADP & NADH.
   c. Enzyme in some samples may interfere with the assay. Enzymes in samples can be removed by using 10 kD spin column.

2. **Standard Curve Preparation:** Dilute Acetate Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Acetate to 990 µl dH_2O. Mix well. Add 0, 2, 4, 6, 8 and 10 µl of 1 mM Acetate Standard into series of wells in 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well of Acetate Standard. Adjust final volume to 50 µl/well with Acetate Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
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

Add 50 µl of the Reaction Mix to each well containing the Standard & test samples and 50 µl of Background Control Mix to sample background control well(s). Mix well.
Note: To avoid possible interference from acetate in the air, we recommend covering the plate with the 96 well plate cover.

4. **Measurement:** Incubate at room temperature for 40 minutes and measure OD_{450nm}.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Acetate Standard Curve. **Note:** For samples having ADP or NADH, correct sample background by subtracting the value derived from the background control from sample readings. Apply the corrected sample reading to the Acetate Standard Curve to get B nmol of Acetate amount in the sample.

Sample Acetate concentration = B/V x Dilution Factor = nmol/ml = µM

Where: **B** is the Acetate amount from the Standard Curve (nmol)

V is the sample volume added into the reaction well (ml)

Acetic Acid MW: 60.05 g/mol

Sample Acetate concentration can also be expressed in nmol/mg or µmol/g of sample.

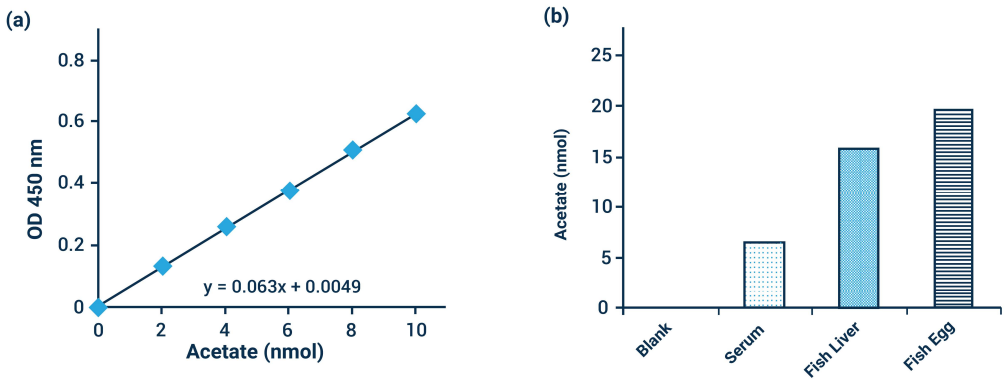

Figure 1. Acetate Standard Curve (a). Measurement of Acetate in human serum (5 µl), fish liver (~100 µg) & egg (~200 µg) (b). Assays were performed following kit protocol.

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