Product Information

Acetate Colorimetric Assay Kit

I. Kit Contents:

Components	K2209-100 100 assays	Cap Color	Part Number
Acetate Assay Buffer	27ml	WM	K2209-C-1
Acetate Enzyme Mix (Lyophilized)	1 vial	Green	K2209-C-2
ATP (Lyophilized)	1 vial	Orange	K2209-C-3
Acetate Substrate Mix (Lyophilized)	1 vial	Blue	K2209-C-4
Probe (Lyophilized)	1 vial	Red	K2209-C-5
Acetate Standard (Lyophilized)	1 vial	Yellow	K2209-C-6

II. Introduction:

Acetate is an important molecule for biosynthesis. In living organisms, acetate is used to generate acetyl coenzyme A (acetyl-CoA), which is central to metabolism of fats and carbohydrates. Acetyl-CoA is also involved in the synthesis of neurotransmitter acetylcholine. Acetylation/deacetylation of proteins is important for post-translational regulation of their functions.

The Acetate Colorimetric Assay Kit provides a sensitive, simple and fast way for detection of acetate levels in various samples based on colorimetric method. In the assay, in the presence of Acetate Enzyme Mix and Acetate Substrate Mix, acetate is converted to an intermediate, which then reduces a colorless probe to a colored product with strong absorbance at 450 nm. The kit can detect less than 20 µM acetate in various samples.

III. Application:

Measurement of Acetate in various tissues/cells.

Analysis of metabolism and cell signaling in various cells.

VI. Sample Type:

Serum & plasma.

Animal tissues: Liver, kidney, muscle, heart etc.

Cell culture: Adherent or suspension cells.

Food.

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℃, 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℃. 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℃. 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℃.

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℃.

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℃. 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.

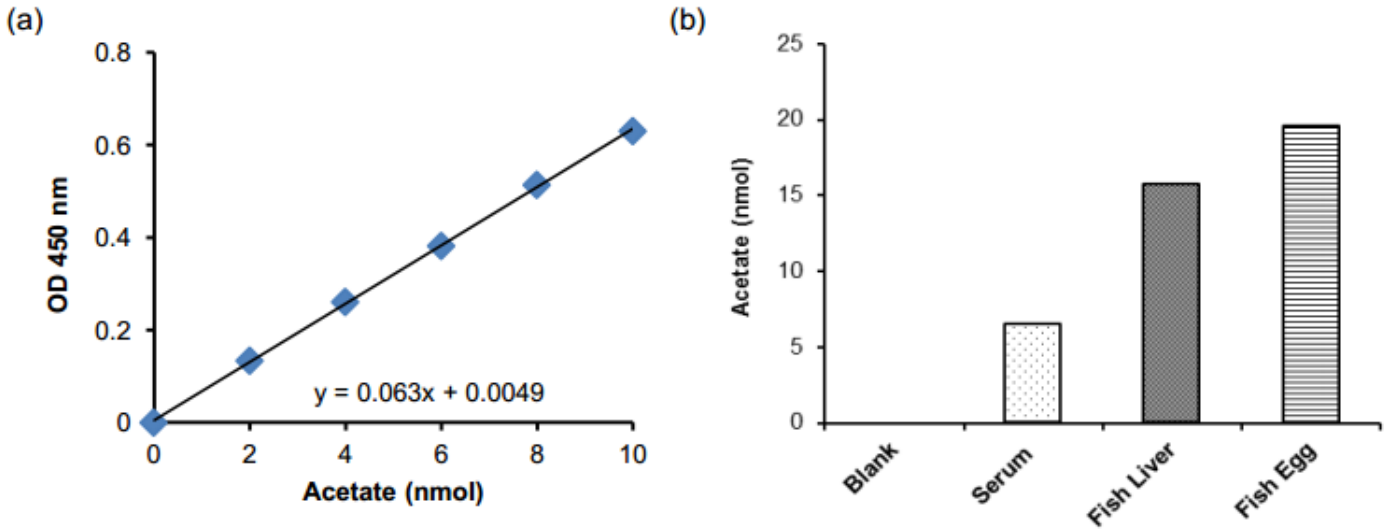

Figure 1. Acetate Standard Curve (a). Measurement of Acetate in human serum (5 μl), fish liver (~100 μg) & egg (~200 μg) (b). Assays were performed following kit protocol.

Frequently Asked Questions

1. We see very high background values and hence the numbers are negative after subtraction. What is the solution?

Acetate vapors absorbed into the reagents can cause high background. Absorption of vapors is a common issue and this can destabilize the kit components. Special care needs to be taken to prevent this.

2. Is EDTA usable for blood collection for this assay?

One or more of the enzymes in the kit requires divalent metal cations. Hence EDTA in samples is not advisable for this assay.

3. Does the kit measure free or bound acetate?

The kit can be used to measure free Acetate in biological samples. If the acetyl group is bound to protein or any other chemical moiety while in solution, it cannot be measured.

4. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

5. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

6. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, it is strongly recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com