



Acetate Kinase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1135

(Version 2.1B)

Detection and Quantification of Acetate Kinase Activity in Tissue
extracts, Cell lysate, Cell culture media and Other biological
fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Acetate kinase (EC 2.7.2.1), which is predominantly found in micro-organisms, facilitates the production of acetyl-CoA by phosphorylating acetate in the presence of ATP and a divalent cation.

Acetate Kinase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Acetate kinase activity in various samples. Acetate kinase activity is determined by NADH decomposition rate. The reaction products can be measured at a colorimetric readout at 450 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Substrate Diluent	4 mlx 1	4 °C
Enzyme	Powder x 1	-20 °C
Enzyme Diluent	1.5 mlx 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	10 mlx 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L stock standard solution. Store stock solution at -20 °C for 2 weeks. Add 0.1 ml stock solution into 0.4 ml distilled water, the concentration will be 400 µmol/L as top standard solution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5 µmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Enzyme Diluent to dissolve before use. Store at -80 °C for 1 month.

Substrate: Briefly centrifuge prior to opening. Add 4 ml Substrate Diluent before use. Store at -20 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 0.5 ml Enzyme Diluent to dissolve before use. Store at -80 °C for 1 month.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5 ml distilled water to dissolve before use, mix, store at 4 °C for 1 month after reconstitution.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect it directly.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	80 μ l	80 μ l	80 μ l	--	--
Distilled water	--	10 μ l	--	40 μ l	140 μ l
Sample	10 μ l	--	--	--	--
Positive Control	--	--	10 μ l	--	--
Enzyme	10 μ l	10 μ l	10 μ l	--	--
Substrate	40 μ l	40 μ l	40 μ l	--	--
Mix and incubate at 37 for 15 minutes					
Standard	--	--	--	100 μ l	--
Dye Reagent A	50 μ l	50 μ l	50 μ l	50 μ l	50 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix and incubate at room temperature for 2 minutes, record absorbance measured at 450 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One Unit of Acetate Kinase activity is defined as the enzyme reduces 1 μmol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the quantity of cells

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

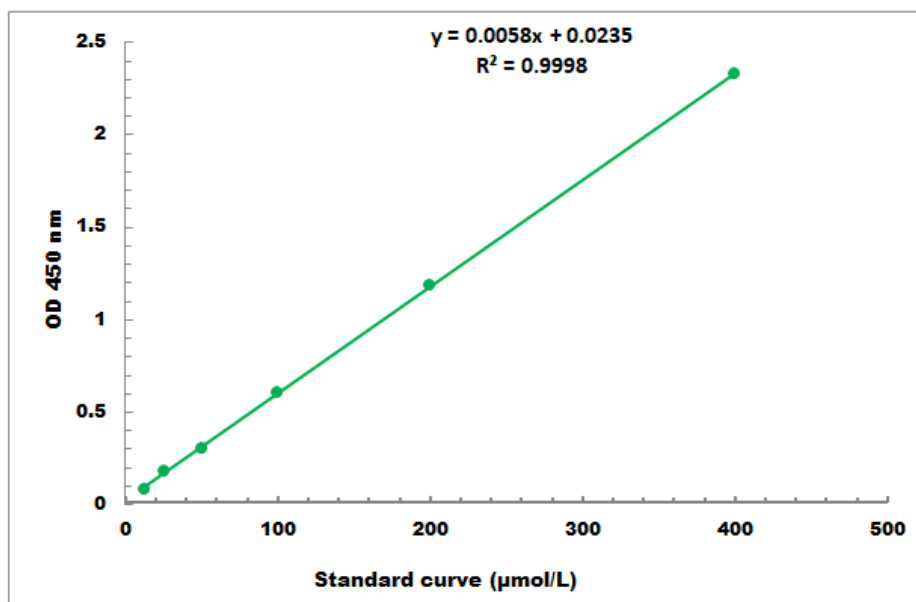
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

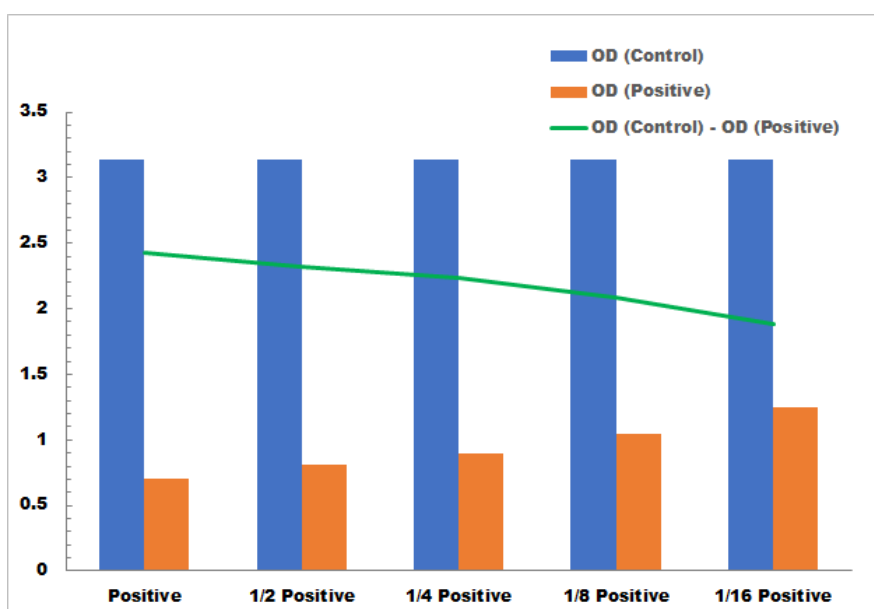
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration