



# **Acetate Kinase Microplate Assay Kit**

## **User Manual**

**Catalog # CAK1135**

(Version 1.3A)

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 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 340 nm.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Enzyme	40 µl x 1	-20 °C
Standard	Powder x 1	-20 °C
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### Note:

**Substrate:** add 18 ml Diluent to dissolve before use.

**Enzyme:** add 1 ml Diluent to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

#### IV. 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  $5 \times 10^6$  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

Add 0.9 ml Assay buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

## V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 $\mu$ l	--
Distilled water	--	--	200 $\mu$ l
Enzyme	10 $\mu$ l	--	--
Substrate	180 $\mu$ l	--	--
Sample	10 $\mu$ l	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

**Unit Definition:** One Unit of Acetate Kinase activity is defined as the enzyme reduces 1  $\mu\text{mol}$  of NADH per minute.

### 1. According to the protein concentration of sample

$$\begin{aligned} \text{AK (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned} \text{AK (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 4 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

### 3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{AK (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 4 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N \end{aligned}$$

### 4. According to the volume of serum or plasma

$$\begin{aligned} \text{AK (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} / T \\ &= 4 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L}$  = 0.4  $\mu\text{mol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l}$  = 0.2 ml;

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

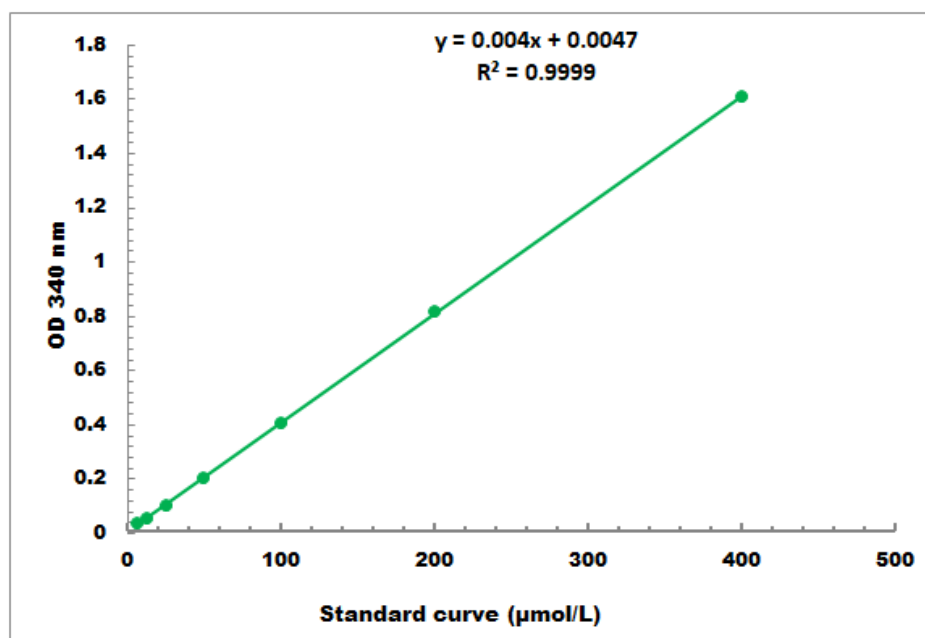
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

## VII. TYPICAL DATA

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



Detection Range: 4 μmol/L - 400 μmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES