Adenylate Kinase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1331

(Version 1.1A)

Detection and Quantification ofAdenylate KinaseActivityinTissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Adenylate kinase (EC 2.7.4.3) is a key enzyme in cellular energy metabolism that catalyzes the reversible transfer of a phosphate group between adenine nucleotides, maintaining the homeostasis of the ATP, ADP, and AMP pools.

Adenylate Kinase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining adenylatekinase activity in various samples. Adenylatekinase 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-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	8 mlx 1	4 °C
Diluent	4 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Enzyme	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 mlx 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. Dissolvein 1 mldistilled water to generate 2mmol/Lstock standard solution. Store stock solution at -20 °C for 2 weeks.Add 0.1 ml stock solution into 0.4 mldistilled water, the concentration will be 400 µmol/L as top standardsolution. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25µmol/L.

Substrate: Briefly centrifuge prior to opening.Add1ml distilled water before use.Keep in dark and store at 4 °C for 1-2 days or -20 °C for 2 weeks.

Coenzyme: Briefly centrifuge prior to opening. Add1ml Diluent before use. Keep in dark and store at 4 °C for 1-2 days or -20 °C for 2 weeks.

Enzyme: Briefly centrifuge prior to opening. Add1 ml Diluent to dissolve before use.Store at -80 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Dissolvein 1 mlDiluent to generatePositive Control (10X).Store at -80 °C for 1 month. Add 0.1 ml Positive Control (10X)into 0.9 mlDiluentto prepare Positive Control (1X) before use.

Dye Reagent A: Briefly centrifuge prior to opening. Add 7 ml distilled water to dissolve before use, mix, store at 4°C for 1 monthafter reconstitution.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 mlAssay 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 regents to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl	--	--
Distilled water	--	10 µl	--	20 µl	120 µl
Sample	10 µl	--	--	--	--
1×Positive Control	--	--	10 µl	--	--
Coenzyme	10 µl	10 µl	10 µl	--	--
Enzyme	10 µl	10 µl	10 µl	--	--
Substrate	10 µl	10 µl	10 µl	--	--
Mix andincubate at 37 for 15 minutes					
Standard	--	--	--	100 µl	--
Dye Reagent A	70 µl	70 µl	70 µl	70 µl	70 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix andincubate 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 ofAdenylate Kinase activity is defined as the enzyme reduces 1 μmolof NADH per minute.

1. According to the slope of the standard curve

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

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

2.1According to the quantity of cells

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

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, μmol/mL

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

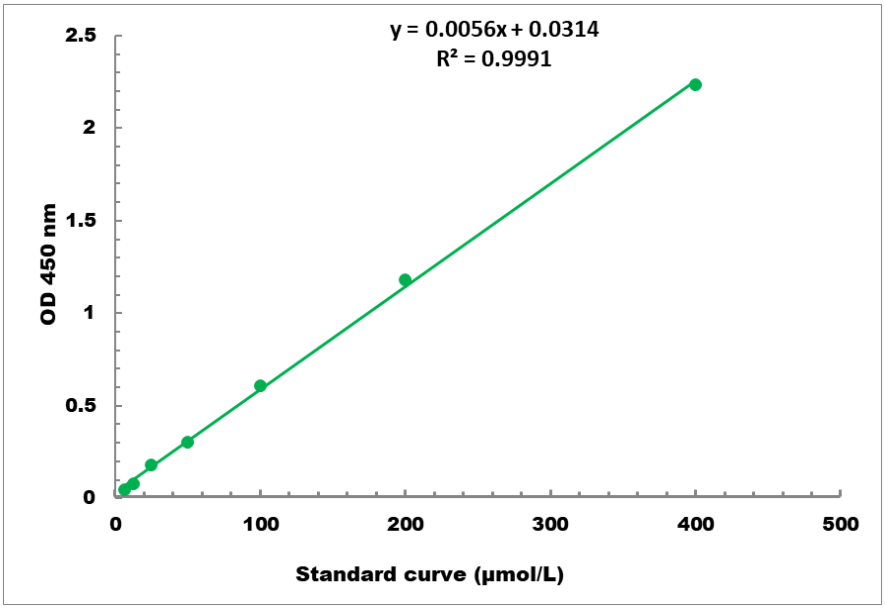
V_{Sample}: the volume of sample in assay procedure, mL

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

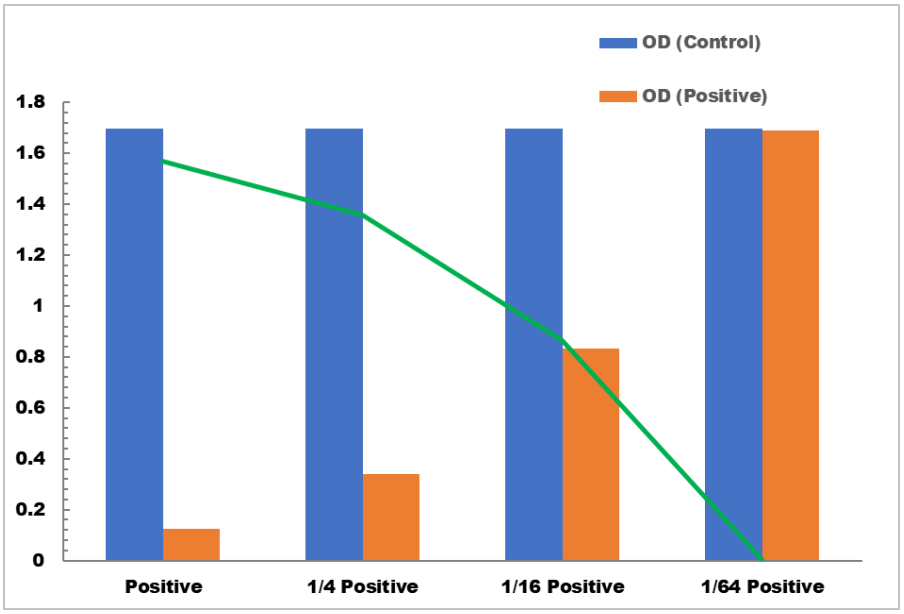
T: the reaction time, minute

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