ATPase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1329

(Version 1.1A)

Detection and Quantification of ATPaseactivity inTissue extracts,

Cell lysate samples.

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

I. INTRODUCTION……………………………………………………………………………………2
II. KIT COMPONENTS……………………………………………………………………………………3
III. MATERIALS REQUIRED BUT NOT PROVIDED……………………………………………………4
IV. REAGENT PREPARATION……………………………………………………………………………5
V. SAMPLE PREPARATION………………………………………………………………………………6
VI. ASSAY PROCEDURE…………………………………………………………………………………7
VII. CALCULATION………………………………………………………………………………………8
VIII.TYPICAL DATA………………………………………………………………………………………9

I. INTRODUCTION

ATPase is a crucial biological catalyst that hydrolyzes ATP to release energy, powering various cellular processes such as ion transport, biosynthesis, and muscle contraction. It serves as a core molecular machine for cellular energy metabolism and signal transduction.

ATPase ActivityColorimetricMicroplate Assay Kit provides a convenient tool for sensitive detection of ATPase activity in a variety of samples. In this assay, ATPase hydrolyzes ATP releasing ADP and a free phosphate ion. Then the dye reagent forms a color with released phosphate ion, which is measured on a plate reader 650 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay BufferI	25 ml x 2	4 °C
Assay Buffer II	20 ml x 1	4 °C
SaturatedAmmonium SulfateSolution	21 ml x 2	RT
Substrate	Powder x 1	-20 °C, keep in dark
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent A	8 ml x 1	4 °C, keep in dark
Dye Reagent B	10 ml x 1	-20 °C, keep in dark
Dye Reagent C	2.5 ml x 1	4 °C
Standard (1 mM)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note: Avoid freeze-thaw cycles

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 650 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Refrigerated High-Speed Centrifuge(4°C,>10,000 g)

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be1000/500/250/125/62.5/31.25/15.625μmol/L.

Substrate Working Solution: Briefly centrifuge prior to opening.Dissolvein 0.5 ml distilled water to generate 5×Substrate stock solution.Store at 4 °C for 2 weeks or -20°C for 2-3 months.Then dilute to1×Substrateworking solutionby adding 20 μl stock solution into 80μl distilled water**.**Prepare immediately and use within 24 hours.

Dye ReagentWorking Solution: PrepareDye Reagent Working Solution by mixing Dye Reagent A, Dye Reagent B and Dye Reagent C immediately before the test. Prepare Dye Reagent Working Solution for immediate use. Store the unused solution in dark at 4°C for at most 24 hours.The volumes of reagent could be enlarged according to the specified numbers of well. The table below gives some examples.

No. of wells	Dye Reagent A (μl)	Dye Reagent B(μl)	Dye Reagent C(μl)
1	24	35	11
5	118	175	57
10	236	350	114
50	1181	1750	569
100	2363	3500	1138

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5mlAssay BufferI for 3×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 1000g 4°C for 10minutes, take the supernatant into a new centrifuge tube andcentrifuged at 10000g 4°C for 30minutes. Discard the supernatant and resuspend the pellet by0.1mlAssay BufferII. Keep it on ice for endogenous phosphate remove.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.5mlAssay BufferI on ice, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 1000g 4°C for 10minutes, take the supernatant into a new centrifuge tube andcentrifuged at 10000g 4°C for 30minutes. Discard the supernatant and resuspend the pellet by0.1mlAssay BufferII. Keep it on ice for endogenous phosphate remove.

3. Endogenous phosphate removes

To avoid free phosphate interfere with assay, add 0.415 ml Saturated Ammonium Sulfate solution to 0.1 ml sample resuspendby Assay Buffer IIand place on icefor 20 mins. Spin down samples at 10,000 g at 4°C for 20 mins,discard the supernatant, and resuspend the pellet back by0.1mlAssay BufferII.

VI. ASSAY PROCEDURE

Add following reagents intothe microcentrifuge tubes:

Reagent*	Sample**	Control	Standard	Blank
Reaction Buffer	110µl	110µl	110µl	110µl
Substrate Working Solution	10µl	10µl	--	--
Distilled water	--	10µl	--	20 µl
Sample	10µl	--	--	--
Mix, room temperature for30 minutes.				
Standard	--	--	20 µl	--
Dye ReagentWorking Solution	70 µl	70 µl	70 µl	70 µl
Mix, room temperaturefor 5 minutes, record absorbance measured at 650nm.				

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 ATPaseactivityis defined asthe enzyme generates 1μmol of PO_4^{3-} per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times V/(V + V_{Assay})\times T}(U/mg)$$

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, μmol/mL

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

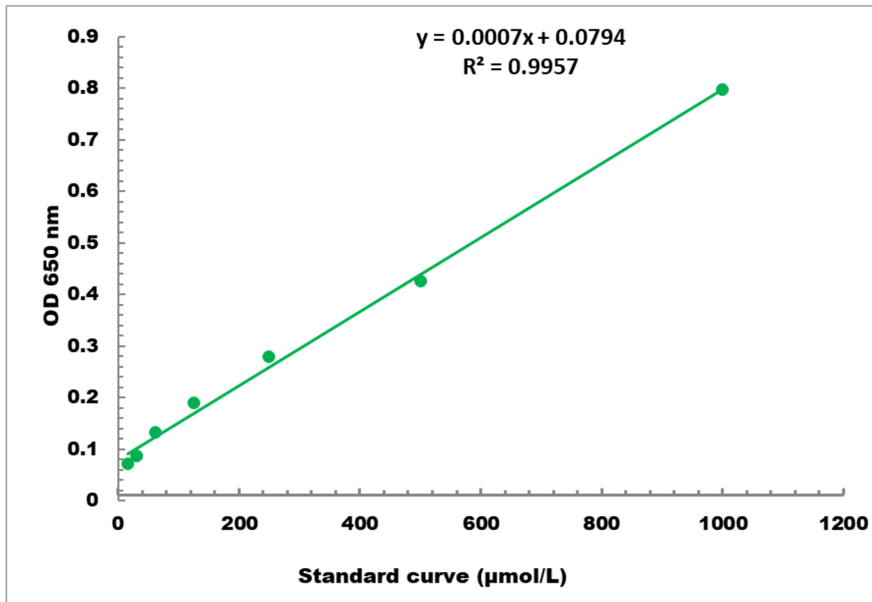
V_{Sample}: the volume of sample in assay procedure, mL

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

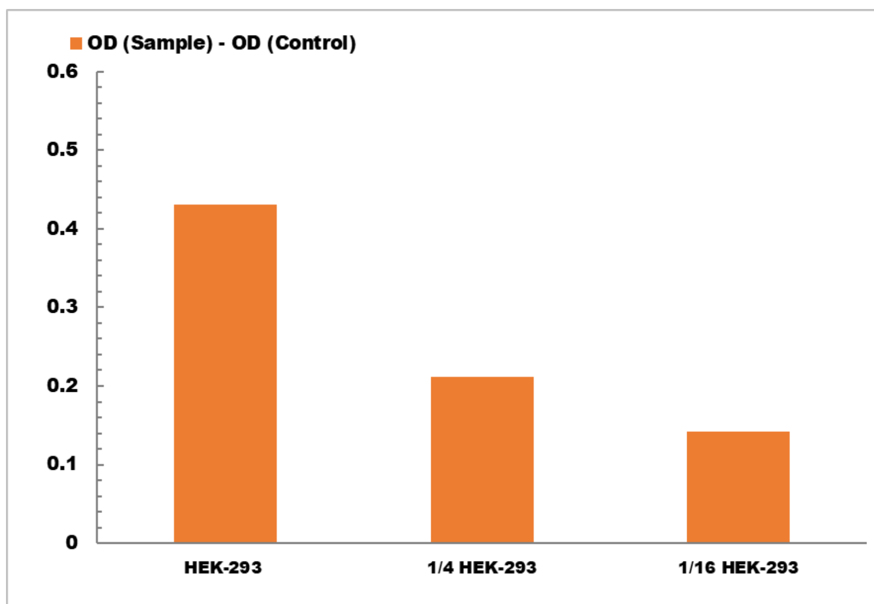
T: the reaction timebefore adding Dye Reagent,minute

VIII. TYPICAL DATA

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



Detection Range: 10μmol/L - 1000μmol/L



Determination of ATPase in membrane fraction of HEK-293 cell line