ATP

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1120

(Version 1.5B)

Detection and Quantification ofATPcontentinUrine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

ATP (Adenosine 5'-triphosphate) is the chemical energy for cellular metabolism and is often referred to as “energy currency" of the cell. ATP is produced only in living cells during photosynthesis and cellular respiration and consumed in cellular processes including biosynthetic reactions, motility and cell division. It is a key indicator of cellular activity and has been utilized as a measure of cell viability and cytotoxicity in research and drug discovery.

ATP ColorimetricMicroplate Assay Kit is a sensitive assay for determining ATP in various samples. ATP concentration is determined by creatine kinase and creatine.The reaction products can be measured at a colorimetric readout at 660 nm.

III.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Reaction Buffer	1 mlx 1	4 °C
Substrate	Powder x 1	-20 °C, keep in dark
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent III	10 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Important Recommendation:*Use disposable plastic tube to avoid phosphorus pollution.*

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 5 mmol/L of standardstock solution. Store at 4 °C for 1 week or -20 °C for 1-2 monthsafter reconstitution.Then dilute to2.5 mmol/Lstandard top solutionby adding 500 µl stock solution into 500µl distilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be2.5/1.25/0.625/0.312/0.156/0.078/0.039mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use.Store at 4 °C for 1-2days or -20 °C for 2 weeks.

Substrate: Add 6 ml distilled waterand heat to dissolve before use.Store at 4 °C for 1 week or -20 °C for 2-3 monthsafter reconstitution.

Dye Reagent: Add 5 ml Dye Reagent III into Dye Reagent I and 1 ml Dye Reagent III into Dye Reagent II respectivelyto dissolve. Transfer allDye Reagent IIinto Dye Reagent III, mix, then transfer all Dye Reagent I into Dye Reagent III (Must follow this step).The mixed Dye Reagent may store at -20 °C for 2-3 weeks.***Note:**It should be yellow. If colorless, the solution is failure. If blue, the solutionispolluted.This solution should be preparedbefore use.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1mlAssay Bufferfor 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times), centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube, and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1mlAssay Buffer,sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times),centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube, and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all thereagents to 37°C before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Substrate	60 µl	60 µl	60 µl
Reaction Buffer	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven,37°Cfor 30 minutes.			
Dye Reagent Working Solution	100 µl	100 µl	100 µl
Mix,room temperaturefor 20 minutes, record absorbance measured at 660 nm.			

Note:

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

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

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

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

V_{Assay}: the volume of Assay Buffer, μl

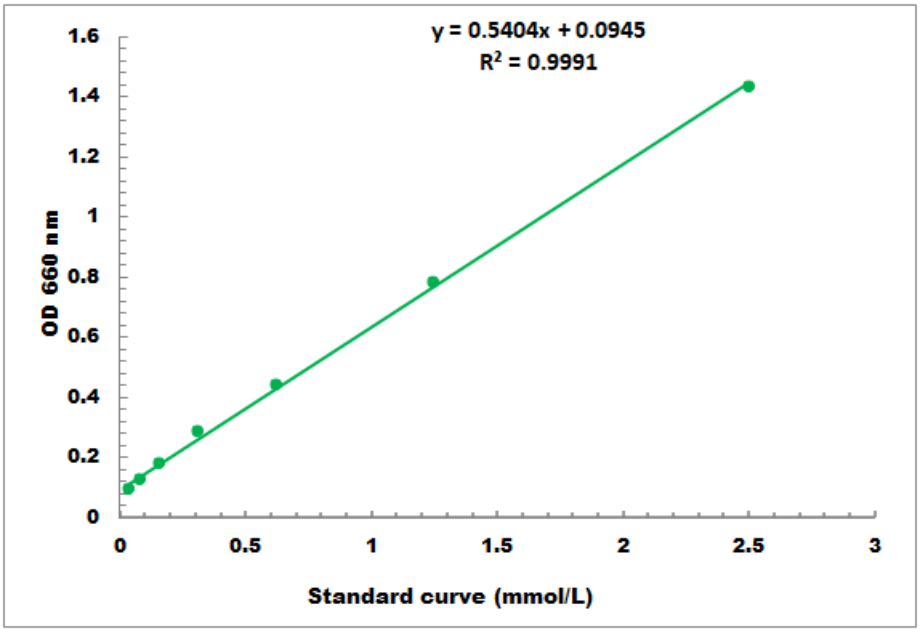
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

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

VIII. TYPICAL DATA

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



Detection Range: 0.02mmol/L -2.5mmol/L