ATP Microplate Assay Kit

User Manual

Catalog # FTA0117

(Version 1.2A)

Detection and Quantification of ATP content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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 Microplate 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 635 nm.

II. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 635 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

III. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Reaction Buffer	1 ml x 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 II Diluent	6 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

It is best to use disposable plastic tube to avoid phosphorus pollution.

Enzyme: add 1 ml distilled water to dissolve before use.

Substrate: add 6 ml distilled water and heat to dissolve before use.

Dye Reagent I: add 4 ml distilled water to dissolve before use.

Dye Reagent II: add 6 ml Dye Reagent II Diluent and heat to dissolve before use.

Dye Reagent Working Solution: add 6 ml Dye Reagent II into 4 ml Dye Reagent I, mix; store at 4 °C for 2-3 days.

Standard: add 1 ml distilled water to dissolve before use, then add 200 µl into 800 µl distilled water, mix; the concentration will be 1 mmol/L.

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml Assay buffer I for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times), centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube; add 0.5 ml Assay buffer II, mix, 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

Weigh out 0.1 g tissue, homogenize with 0.5 ml Assay buffer I, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube; add 0.5 ml Assay buffer II, mix, 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

Add 0.1 ml serum or plasma and 0.5 ml Assay buffer I into the microcentrifuge tube, mix, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube; add 0.5 ml Assay buffer II, mix, centrifuged at 8000g 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 the reagents to 37°C before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Substrate	60 µl	60 µl	60 µl
Reaction Buffer	10 µl	10 µl	10 µl
Enzyme	20 µl	20 µl	20 µl
Mix, put it in the oven, 37 °C for 30 minutes.			
Dye Reagent Working Solution	100 µl	100 µl	100 µl
Mix, room temperature for 10 minutes, record absorbance measured at 635 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

VI. CALCULATION

1. According to the protein concentration of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$ATP\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times V / V_{Assay})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$$

$C_{Standard}$: the standard concentration, 1 mmol/L = 1 μmol/ml;

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

W: the weight of sample, g;

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

V: the volume of serum or plasma, ml;

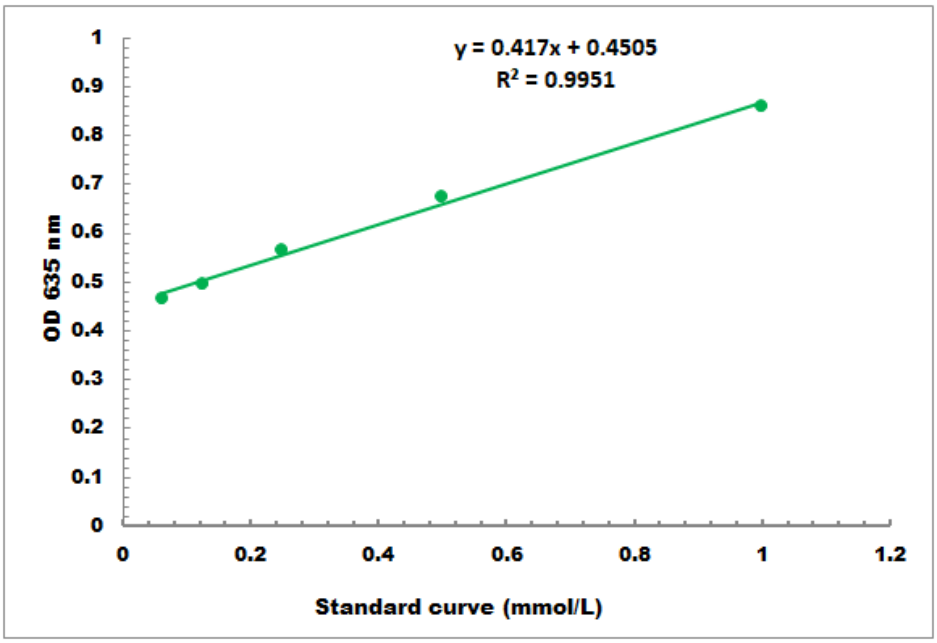
$V_{Standard}$: the volume of standard, 0.01 ml;

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

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 1 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES