ATP Microplate Assay Kit

Cat #: orb707328 (manual)

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.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

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 660 nm.

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

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	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 III	10 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.

Standard: add 1 ml distilled water to dissolve before use, mix; then add 500 µl into 500 µl distilled water, the concentration will be 2.5 mmol/L.

Dye Reagent: add 5 ml Dye Reagent III into Dye Reagent I and 1 ml Dye Reagent III into Dye Reagent II respectively to dissolve. Transfer all Dye Reagent II into 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 4 °C for 2-3 days.

***Note:** It should be yellow. If colorless, the solution is failure. If blue, the solution is polluted. This solution should be prepared before use. It is best to use disposable plastic containers to prepare the solution in order to prevent phosphorus pollution.

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 5×10^6 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

ASSAY PROCEDURE

Warm all the reagents to 37°C before use.

Add following reagents into the 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 °C for 30 minutes.			
Dye Reagent Working Solution	100 µl	100 µl	100 µl
Mix, room temperature for 20 minutes, record absorbance measured at 660 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.

CALCULATION

1. According to the protein concentration of sample

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

$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cells or bacteria

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

$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

4. According to the volume of serum or plasma

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

$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$

$C_{Standard}$: the standard concentration, 2.5 mmol/L = 2.5 μmol/ml;

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

W: the weight of sample, g;

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

V: the volume of serum or plasma, ml;

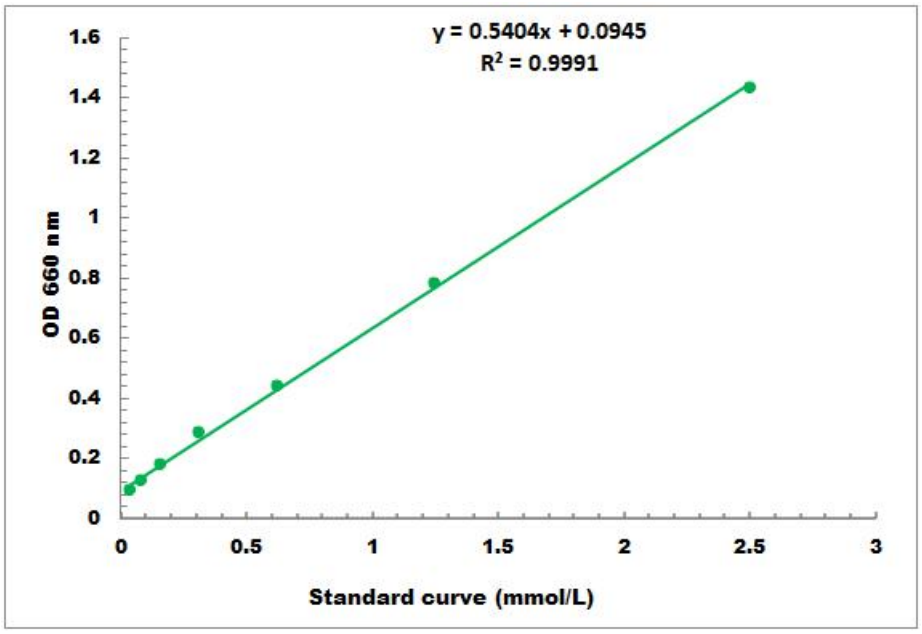
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

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

TYPICAL DATA

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



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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558