ATP Synthase

(Mitochondrial Complex V)

Microplate Assay Kit

User Manual

Catalog # CAK1127

(Version 1.3A)

Detection and Quantification of ATP Synthase (Mitochondrial Complex V) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

ATP synthase (EC 3.6.3.14) is an important enzyme that creates the energy storage molecule adenosine triphosphate (ATP). ATP is the most commonly used "energy currency" of cells for most organisms. It is formed from adenosine diphosphate (ADP) and inorganic phosphate (Pi), and needs energy for its formation.

The assay is used to determine ATP Synthase activity. The enzyme catalysed reaction products Pi can react with dry reagent, and can be measured at a colorimetric readout at 660 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	Powder x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Reaction Buffer	4 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Stop Solution	5 ml x 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent III	15 ml x 1	4 °C
Standard (10 µmol/ml)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Assay Buffer II: add 1.2 ml ethanol to dissolve before use.

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

Dye Reagent: add 10 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.

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. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 11000g 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 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 11000g 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 in the microcentrifuge tubes:

Reagent	Blank	Sample	Standard
Reaction Buffer	40 µl	40 µl	--
Substrate	10 µl	10 µl	--
Sample	--	50 µl	--
Distilled Water	50 µl	--	--
Mix, incubate at 37°C for 30 minutes.			
Stop Solution	50 µl	50 µl	--
Mix, centrifuged at 4,000g, 10 minutes, add the supernatant into the microplate.			
Supernatant	50 µl	50 µl	--
Standard	--	--	50 µl
Dye Reagent	150 µl	150 µl	150 µl
Mix, wait for 10 minutes, measured at 660 nm and record the absorbance.			

Note:

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

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One Unit of Complex V activity is defined as the enzyme produces 1 μmol of Pi per hour.

1. According to the protein concentration of sample

$$\text{Complex V (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / (C_{Protein} \times V_{Sample}) / T \times 3$$

$$= 60 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / C_{Protein}$$

2. According to the weight of sample

$$\text{Complex V (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / (W \times V_{Sample} / V_{Assay}) / T \times 3$$

$$= 12 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / W$$

3. According to the quantity of cells or bacteria

$$\text{Complex V (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / (N \times V_{Sample} / V_{Assay}) / T \times 3$$

$$= 12 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / N$$

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

$C_{Standard}$: the standard concentration, 10 μmol/ml;

W: the weight of sample, g;

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

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.05 ml;

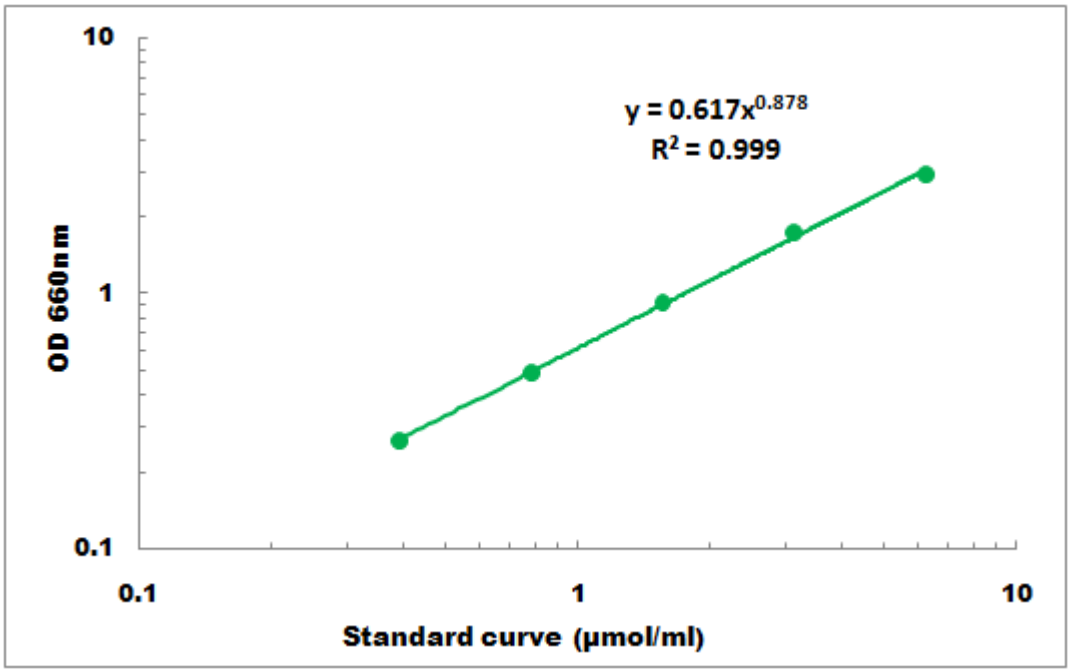
$V_{Standard}$: the volume of standard, 0.05 ml;

V_{Assay}: the volume of Assay buffer, 0.2 ml;

T: the reaction time, 30 minutes = 0.5 hour.

VII. TYPICAL DATA

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



Detection Range: 0.1 µmol/ml - 10 µmol/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES