ATP Synthase

(Mitochondrial Complex V) Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1127

(Version 1.5A)

Detection and Quantification ofATP Synthase (Mitochondrial Complex V)ActivityinTissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

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.

ATP Synthase ActivityColorimetric Microplate Assay Kit provides a sensitive and convenient method for ATP Synthase activity determination.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-WellMicroplate	1 plate	
Assay Buffer I	30 mlx 4	4 °C
Assay Buffer II	Powder x 1	4 °C
Assay Buffer III	20 mlx 1	4 °C
Reaction Buffer	4 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Stop Solution	5 mlx 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent III	15 mlx 1	4 °C
Standard (10 mmol/L)	1 mlx 1	4 °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

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 be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Assay Buffer II: Briefly centrifuge prior to opening. Add 1.2 ml ethanolto dissolve before use. Keep in dark for immediate use or store at -20°C for 3-4 months after reconstitution.

Substrate: Briefly centrifuge prior to opening. Add 1 ml distilled waterto dissolve before use.Prepare for immediate use or store at -20°C for 1 monthafter reconstitution.

Dye Reagent: Add 10 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.It is best to usedisposable plastic containers to prepare the solution in order to prevent phosphoruspollution.

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 0.99mlAssay Buffer Iand 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 198µl 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 10minutes, 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 0.99mlAssay Buffer Iand 10 µl Assay Buffer IIon 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 198µl 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Warm all thereagents 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,10minutes, add the supernatant intothe 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 recordthe absorbance.			

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 ofComplex Vactivity is defined as the enzyme produces 1μmol of Pi per hour.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T}(U/mL)$$

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, mmol/L= μmol/mL

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

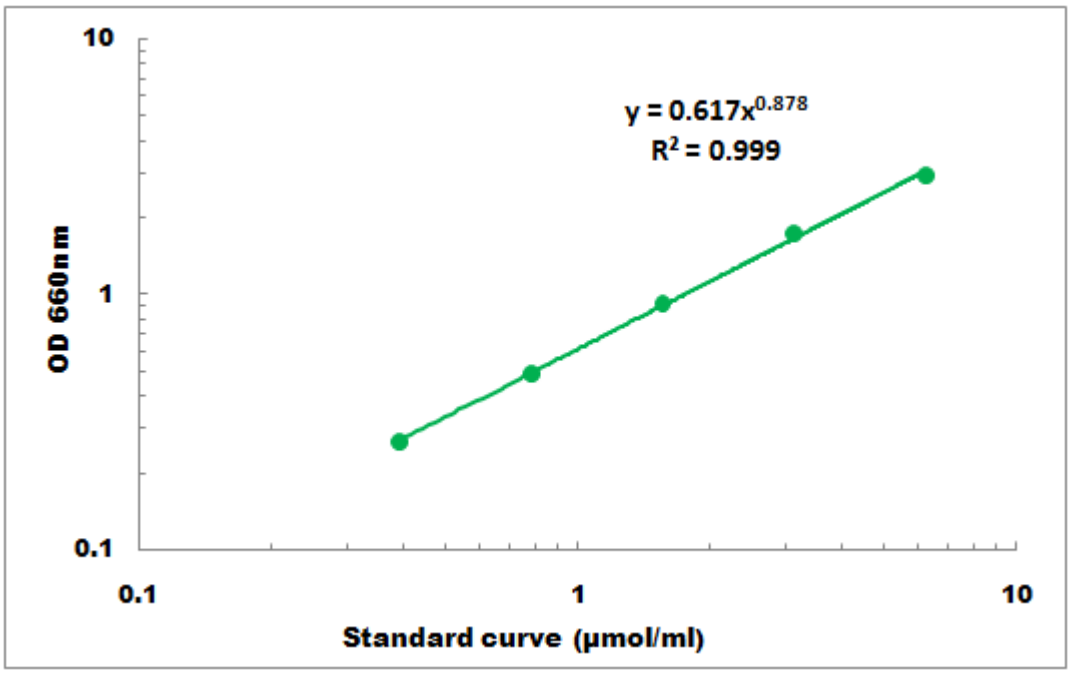
V_{Sample}: the volume of sample in assay procedure, mL

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

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 10 mmol/L