CREATIVE BIOMART®
Assay Kit

ATP Synthase Microplate Assay Kit

Product Information

Cat.No. Kit-3500

Product Overview

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 Cytochrome C oxidase activity. The enzyme catalysed reaction products Pi can react with dry reagent, and can be measured at a colorimetric readout at 660 nm.

Storage

Store at 4 degree C for 6 months.

Size

100 Assays

Kit Components

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

Materials Required but Not Supplied

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

Compatible Sample Types

Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples

Preparation

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.

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%, sonication 3s, intervation 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.

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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%, sonication 3s, intervation 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.

Assay Protocol

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.

Analysis

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
Complex V (U/mg) = (CStandard × VStandard) × (ODSample - ODBlank) / ODStandard / (CProtein × VSample) / T × 3 = 60 × (ODSample - ODBlank) / ODStandard / CProtein
2. According to the weight of sample

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Complex V (U/g) = (CStandard × VStandard) × (ODSample - ODBlank) / ODStandard / (W × VSample / VAssay) / T × 3 = 12 × (ODSample - ODBlank) / ODStandard / W
3. According to the quantity of cells or bacteria
Complex V (U/10^4) = (CStandard × VStandard) × (ODSample - ODBlank) / ODStandard / (N × VSample / VAssay) / T × 3 = 12 × (ODSample - ODBlank) / ODStandard / N
CProtein: the protein concentration, mg/ml;
CStandard: the standard concentration, 10 μmol/ml;
W: the weight of sample, g;
N: the quantity of cell or bacteria, N × 104;
VTotal: the total volume of the enzymatic reaction, 0.2 ml;
VSample: the volume of sample, 0.05 ml;
VStandard: the volume of standard, 0.05 ml;
VAssay: the volume of Assay buffer, 0.2 ml;
T: the reaction time, 30 minutes = 0.5 hour.

Sensitivity
0.1 umol/ml - 10 umol/ml

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