ATP Assay Kit (Spectrophotometry)

Introduction

Adenosine triphosphate (ATP) is widely present in animals, plants, microorganisms, and cultured cells. It functions as a coenzyme that facilitates metabolism and participates in the metabolism of fats, proteins, carbohydrates, nucleic acids, and nucleotides. ATP serves as a primary energy source for biological processes. Cellular energy charge is a key indicator of cellular metabolic status. Measuring ATP content and calculating energy charge provides a direct assessment of energy metabolism.

Product Features

ATP reacts with creatine in the presence of creatine kinase to produce phosphocreatine. The generated phosphocreatine can be quantified at 700 nm using the phosphomolybdate colorimetric method, which reflects the ATP content.

Kit Components

Taking 50T/24S packing for example:

Catalog. No	Packing	Storage
CB0032S-ES-Acidic	25 mL × 1 bottle	4 °C
CB0032S-ES-Basic	25 mL × 1 bottle	4 °C
CB0032S-A	Powder × 1 vial; dissolved in 3.5 mL distilled water before use; aliquot remaining solution and store at –20 °C; avoid repeated freeze-thaw	4 °C
CB0032S-B	3 mL × 1 vial	4 °C
CB0032S-C	Powder × 1 vial; dissolve in 600 µL distilled water before use; aliquot remaining solution and store at –20 °C; avoid repeated freeze-thaw	4 °C
CB0032S-D	10 mL × 1 bottle	4 °C
CB0032S-E	50 mL × 1 bottle	4 °C
CB0032S-S	1 mL × 1 vial, 0.5 µmol/mL ATP standard solution	4 °C

Note: Perform a pretest with 2–3 representative samples before formal measurement.

Instruction

1. **Preparation of Lab Instruments**

   Spectrophotometer or microplate reader, Water bath, Adjustable micropipettes, 1 mL glass cuvettes, Mortar and pestle, Distilled water.

2. **ATP Extraction**

   From serum or plasma:

   Mix sample with distilled water at a 1:5–10 ratio (e.g., 0.1 mL serum + 1 mL water), homogenize on ice, heat at 100 °C for 5 min, then centrifuge at 8000 g, 4 °C for 15 min. Collect the supernatant for assay.

   From tissues:

   Mix tissue with distilled water at a 1:5–10 ratio (e.g., 0.1 g tissue + 1 mL water), homogenize on ice, heat at 100 °C for 5 min, then centrifuge at 8000 g, 4 °C for 15 min. Collect the supernatant.

From cells or bacteria:

Collect cells or bacteria by centrifugation, discard supernatant. Add distilled water at 500–1000:1 ratio relative to cell/bacterial number (e.g., 5×10^6 cells in 1 mL water), lyse by sonication on ice (1 min, 20% amplitude or 200 W, 2s pulse / 1s pause), then centrifuge at 8000 g, 4 °C for 15 min. Collect the supernatant.

3. Assay Procedure

1) Preheat spectrophotometer or microplate reader for at least 30 min, set wavelength to 700 nm, and zero with distilled water.
2) Prepare the color reagent immediately before use: mix CB0032S-D and CB0032S-E at 1:5 ratio according to the number of samples (0.87 mL per sample).
3) Sample measurement (add reagents in EP tubes):

Reagent	Test Tube (μL)	Control Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample	30	30	-	-
Standard solution	-	-	30	30
CB0032S-A	60	-	60	-
CB0032S-B	30	30	30	30
CB0032S-C	30	-	30	-
Distilled water	-	90	-	90
Mix thoroughly, incubate in a 37 °C water bath for 30 min.				
Color reagent	600	600	600	-
Incubate at 37 °C for 20 min, and measure absorbance at 700 nm.				

Note: Typically, one or two replicates for blank and standard tubes are sufficient.

4. Calculation

1) Serum or plasma:

ATP (μmol/mL)=[Cstd × (Att−Ablk) ÷ (Astd −Ablk) ×V1]÷ (V3×V1÷V2) =5×(Att −Ablk) ÷ (Astd−Ablk)

2) Tissue, bacteria, or cells:

By protein concentration:

ATP (μmol/mg prot)=[Cstd × (Att-Ablk)÷(Astd-Ablk)×V1]÷(V1÷Cpr)=0.5×(Att-Ablk)÷(Astd-Ablk)÷Cpr

By fresh weight:

ATP (μmol/g tissue)=[Cstd × (Att-Ablk)÷(Astd-Ablk)×V1]÷(W×V1÷V2)=0.5×(Att-Ablk)÷(Astd-Ablk)÷W

By cell/bacterial number (per 5×10^6 cells):

ATP (μmol/10^4 cell)=[Cstd × (Att-Ablk)÷(Astd-Ablk)×V1]÷(500×V1÷V2)=0.001×(Att-Ablk)÷(Astd-Ablk)

Note:

Cstd: standard solution concentration (0.5 μmol/mL)

Att: A test tube

Ablk: A blank tube

Astd: A standard tube

V1: sample volume in reaction (0.03 mL)

V2: extraction volume (1 mL)

V3: serum/plasma volume (0.1 mL)

Cpr: protein concentration (mg/mL)

W: sample weight (g)

500: total number of cell or bacterial (5million).

Precautions

1. Detection limit: 10 nmol/mL or 10 nmol/g fresh weight or 0.1 nmol/mg prot.
2. For protein quantification, it is recommended to use TargetMol BCA Protein Quantification Kit (C0050).
3. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
4. Please wear a lab coat and disposable gloves.