CREATIVE BIOMART®
Assay Kit

ATPase Activity Assay Kit (Colorimetric)

Product Information

Cat

Kit-1031

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Description

ATPase (Adenosine Triphosphatase: EC 3.6.1.3) is an important enzyme for maintaining the cell membrane potential, transporting ions and regulating cellular volume. It catalyzes the decomposition of ATP into ADP and a free phosphate ion. The hydrolysis of ATP is highly exergonic releasing energy that is utilized in several cellular processes. There are many classes of ATPases including Na+/K+ -ATPase, H+/K+ -ATPase, Ca2+ -ATPase, etc. The deficiency of mitochondrial ATPase is serious: for example, Na+/K+-ATPase deficiency increases anxiety-related behavior, while Ca2+ -ATPase deficiency leads to exertional muscle pain syndrome. Therefore, accurate detection of ATPase activity is valuable for the diagnosis and mechanistic studies of some of these diseases. ATPase Activity Assay kit provides a quick and easy method for monitoring ATPase activity in various samples. In the assay, ATPase hydrolyzes ATP releasing ADP and a free phosphate ion, and through linked reactions, a strong, stable chromophore is generated (OD 650 nm). The assay is simple, sensitive, high -throughput adaptable and can detect ATPase Activity less than 0.005 U/L.

Applications

Measurement of ATPase activity in various tissues/cells
Analysis of energy-generating pathways
Analysis of Na+/K+ -ATPase mediated signal transduction pathways, e.g. MAPK, ROS etc.

Storage

Store kit at -20°C, protected from light. Centrifuge vials briefly, prior to use.
ATPase Assay Buffer: Warm to room temperature before use. Store at 4°C.
ATPase Substrate: Reconstitute one well with 110 µl dH2O. Pipette up and down to dissolve. Aliquot and store at -20°C. Use within two months.
ATPase Developer: Ready to use as supplied and keep it at room temperature.
ATPase Positive Control: Reconstitute with 100 µl Assay Buffer and mix thoroughly. Keep on ice while

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Kit Components

- ATPase Assay Buffer, 25 ml
- ATPase Substrate, 2 vials
- ATPase Developer, 3 ml
- Phosphate Standard (10 mM), 0.5 ml
- ATPase Positive Control, 1 vial

Materials Required but Not Supplied

96-well clear plate with flat bottom
Multi-well spectrophotometer (ELISA reader)

Compatible Sample Types

Animal tissues: Liver, heart, kidney, etc.
Cell culture: Adherent or Suspension Cells

Preparation

1. Sample Preparation: For whole cells or tissue lysate, rapidly homogenize tissue (40 mg) or cells (2 x 10^6) with 400 µl ice cold ATPase Assay Buffer, and place sample on ice for 10 minutes. Centrifuge at 10,000 x g at 4°C for 10 min and collect the supernatant. Important: The phosphate in tissue samples and cell lysates will interfere with assay. Remove endogenous phosphate by using ammonium sulfate method: Aliquot the tissue samples (100 µl) to a clean centrifuge tube, add saturated ammonium sulfate to a final concentration of 3.2 M and place on ice for 20 mins. Spin down samples at 10,000 g at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume. Add samples (2-20 µl) in duplicates onto a clear 96-well plate (labeled "background control", and sample ATPase activity"). For reagent control: add 100 µl ATPase Assay Buffer. Adjust final volume to 100 µl with ATPase Assay Buffer. For ATPase Positive Control: dilute 10 µl of ATPase Positive Control into 190 µl of ATPase Assay Buffer. Add 2-20 µl of ATPase Positive Control into wells and adjust final volume to 100 µl with ATPase assay buffer.
Note: •
For unknown samples, we suggest testing several volumes to ensure the readings are within the

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standard curve range. •
Many detergents commonly found in laboratories contain high amounts of phosphates which can adhere to clean glassware. It is highly recommended to use disposable plastic labware for all samples, standards and reagents to avoid contamination.

Assay Protocol

2. Phosphate Standard Curve: Dilute 10 µl of the 10 mM Phosphate Standard into 990 µl dH2O, mix well to generate 100 µM working Phosphate Standard. Add 0, 10, 20, 30, 40 and 50 µl of 100 µM Phosphate Standard to individual wells to generate 0, 1, 2, 3, 4 and 5 nmol/well of Phosphate Standard. Adjust volume to 200 µl/well with ATPase Assay Buffer.
3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
ATPase Assay Buffer	98 µl	100 µl
ATPase Substrate	2 µl	----

Add 100 µl of the Reaction Mix to each well containing the Positive Control, Reagent Control** and test samples. Incubate at 25°C for 30 min. Do not add Reaction Mix to the Standards.
Note:
*For sample background control, add 100 µl of Background Control mix to each well and mix well.
** For Reagent Control, prepare a well adding 100 µl ATPase Assay Buffer (see step 1).
4. Measurement: Add 30 µl ATPase Assay Developer to all standards, ATPase Positive Control and Test Samples and Sample Background Controls. Incubate at 25°C for 30 min and measure OD at 650 nm in Endpoint mode (i.e., at the end of incubation time).

Analysis

5. Calculation: Subtract the 0 standard reading from all standard readings. Plot the phosphate standard curve. Correct sample background by subtracting the higher value derived from the background control or reagent control from all sample readings (Experimental results indicated that reagent background control shows higher absorbance values). Calculate the ATPase activity of the test sample: $\Delta OD = A2 - A1$. Apply the ΔOD to the Phosphate standard curve to get B nmol of phosphate generated by ATPase during the reaction time (e.g. t = 30 min).

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Sample ATPase Activity = B/(tX V) x D = nmol/min/µl = mU/µl = U/ml
Where: B is the Phosphate amount from standard curve (nmol)
t is the reaction time (min)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor
Unit Definition: One unit of ATPase is the amount of enzyme that will generate 1.0 µmol of phosphate per min at pH 7.5 at 25°C.

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