ATP Assay Kit
(Colorimetric/Fluorometric)

Catalog Number KA0806

100 assays

Version: 05

Intended for research use only

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Introduction

Background

ATP is the primary energy currency of living systems. Virtually all energy requiring processes utilize the chemical energy stored in the phosphate bond of ATP. ATP is formed exclusively in the mitochondria and a variety of genetic diseases can affect ATP formation in the mitochondria. There are a number of commercially available ATP assays which detects femtomoles or less of ATP by measuring luminescence but these kits require specialized luminescence instrumentation and utilize luciferase which can be difficult to maintain in active form. Abnova's ATP Assay Kit (Colorimetric/Fluorometric) is designed to be a robust, simple method which utilizes the phosphorylation of glycerol to generate a product that is easily quantified by colorimetric (λmax = 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The assay can detect down to 50 pmol (1 µM) of ATP in various samples. The kit provides sufficient reagents for 100 assays.

General Information

Materials Supplied

List of component

Component	Amount
ATP Assay Buffer	25 ml
ATP Probe (in DMSO)	0.2 ml
ATP Converter	1 vial
Developer Mix (lyophilized)	1 vial
ATP Standard (1 µmol; lyophilized)	1 vial

Storage Instruction

Store kit at -20℃, protect from light. Briefly centrifuge all small vials prior to opening. Read entire protocol before performing the assay.

Assay Protocol

Reagent Preparation

- ✓ ATP Assay Buffer: Warm to room temperature before use. Store at -20℃ or 4℃.
- ✓ ATP Probe: Ready to use as supplied. Warm to room temperature before using to melt frozen DMSO. Store at -20℃, protect from light and moisture. Use within two months.
- ✓ ATP Converter: Dissolve with 220 µl ATP Assay Buffer. Aliquot and store at -20℃. Use within two months.
- ✓ Developer Mix: Dissolve in 220 µl ATP Assay Buffer. Aliquot and store at -20℃. Use within two months.
- ✓ ATP Standard: Dissolve in 100 µl of distilled water to generate 10 mM stock solution. Keep cold while using. Store at -20℃.

Sample Preparation

Lyse 1 x 10^6 cells or homogenize tissues (10 mg) in 100 µl ATP Assay Buffer. Deproteinize cell lysate or tissue homogenate using deproteinization sample preparation kits or 10 kDa Spin Column. Add 2-50 µl of sample to a 96-well plate. Adjust the volume to 50 µl/well with ATP Assay Buffer.

Notes:

1. *As ATP is labile, for more accurate assays, we recommend using fresh samples. For samples to be assayed at later date, snap freeze samples using liquid N_2 or dry ice.*
2. *Tissues samples may contain enzymes that consume ATP rapidly. We suggest quick processing of your samples and deproteinization using Deproteinization sample preparation kit.*
3. *For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Cure range.*
4. *For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.*
5. *Endogenous compounds may interfere with the reaction. To ensure accurate determination of ATP in the test samples, we remcommend spiking samples with a known amount of Standard (300 pmol).*

Assay Procedure

1. Standard Curve Preparations

   For the colorimetric assay, dilute 10 µl of the ATP Standard with 90 µl of dH_2O to generate 1 mM. ATP standard, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells and adjust volume to 50 µl/well with ATP Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of ATP Standard. For the fluorometric assay, further dilute the ATP Standard to 0.01-0.1 mM with the dH_2O (Detection sensitivity is 10-100 fold higher with the fluorometric than with the colorimetric assay). Follow the procedure as the colorimetric assay.

2. Reaction Mix:

Mix enough reagents for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix:

	Colorimertric Assay	Fluorometric Assay
ATP Assay Buffer	44 µl	45.8 µl
ATP Probe	2 µl	0.2 µl*
ATP Converter**	2 µl	2 µl
Developer	2 µl	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the ATP Standard and test samples.

*Notes: *For the fluorometric assay, use 1/10 of the probe to reduce fluorescence background.*

***Glycerol phosphate generates background. If significant amount of glycerol phosphate is suspected in your sample, a glycerol phosphate background control may be performed by replacing the 2 µl ATP converter with 2 µl of ATP Assay Buffer. In the absence of ATP converter, the assay detects only glycerol phosphate, but not ATP. The glycerol phosphate background should be subtracted from ATP reading.*

3. Measurement: Mix well. Incubate at room temperature for 30 min., protected from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em=535/587 nm) in a micro-plate reader. The signals are stable for over two hrs.

Data Analysis

Calculation of Results

Calculation: Correct background by subtracting the value derived from the 0 ATP standard from all standard and sample readings. If the background control reading is significant, subtract the background control eading from sample reading. Plot the Standard Curve. Apply ATP sample readings to the standard curve to get B nmol of ATP in the sample well.

Sample ATP concentration (C) = B/V x D= nmol/µl or µmol/ml or mM

Where: B is ATP amount in the reaction well from standard curve (nmol).
V is the sample volume added into sample wells (µl).
D is the dilution factor

Note: For spiked samples, correct for any interference by subtracting the background control from spiked samples. Calculate the amount of ATP in the sample readings:

$$\text{ATP amount in sample well (B)}=\left(\frac{(OD_{sample(corrected)})}{(OD_{sample+ATP\ Std(corrected)})-(OD_{sample(corrected)})}\right)$$

ATP molecular weight: 507.18 g/mol

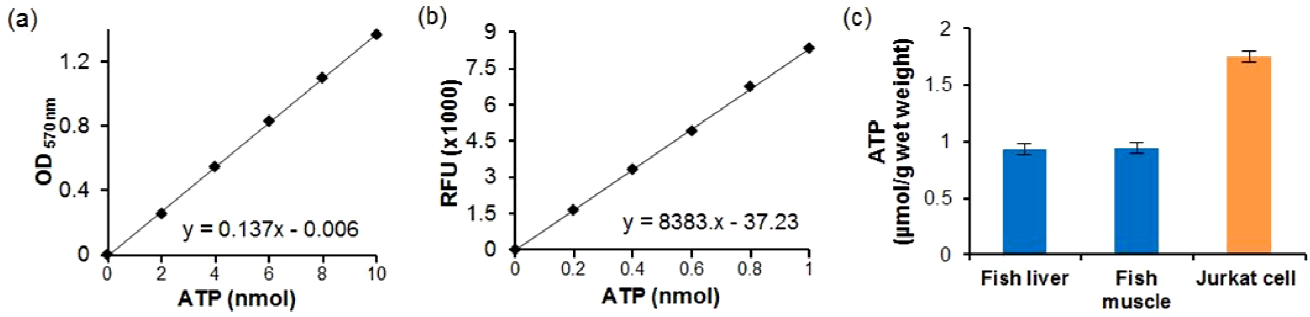

Figure. ATP Standard Curves. a) Colorimetric. b) Fluorometric. c) Quantitation of ATP in fish liver (2.5 µl of 10 times diluted sample), fish muscle (5 µl of 10 times diluted sample) and Jurkat cell lysate (5 µl) using fluorometric assay. Samples were spiked with known amounts of ATP (300 pmol). Assays were performed according to the kit protocol.