ATP Assay Kit (Colorimetric/Fluorometric)

Introduction

ATP is the most crucial energy source within cells, playing a vital role in various cellular processes. Abnormal changes in ATP levels are often associated with impaired cellular functions, making the detection of ATP levels highly important.

This kit provides a simple and sensitive method for quantifying ATP in tissues, cells, and other samples. Glycerol kinase can phosphorylate glycerol using ATP as the phosphate donor, producing glycerol-3-phosphate. Subsequently, glycerol-3-phosphate is oxidized by glycerol phosphate oxidase, generating hydrogen peroxide (H_2O_2). In the presence of horseradish peroxidase, the produced H_2O_2 reacts with the highly sensitive Amplex Red probe to form the fluorescent red product, resorufin. The fluorescence intensity and absorbance of resorufin are directly proportional to the ATP concentration in the sample. Consequently, this kit offers flexible detection, allowing measurements by either colorimetric or fluorometric methods.

Components and Storage

Components \ Size	100 Assays	Storage
ATP Assay Buffer	25 mL	-20°C
Amplex Red	200 µL	-20°C away from light
Enzyme Solution	200 µL	-20°C
Cofactor	200 µL	-20°C away from light
ATP Standard (100 mM)	10 µL	-20°C
Shipping: Blue ice	Shelf life: 1 year	

Protocol

1. Materials required but not supplied

   1) ddH_2O，PBS

   2) Dounce homogenizer (if using tissue)

   For deproteinization procedure

   3) 4 M perchloric acid (PCA)

4) 2 M potassium hydroxide (KOH)

5) 10 kD spin columns (optional)

2. Reagent preparation

1) Warm ATP Assay Buffer and Amplex Red to room temperature before use. Keep Enzyme Solution and Cofactor on ice for use.

2) Dilute ATP Standard (100 mM) in ddH_2O to make an ATP Standard (10 mM). Dilute 10 µL of ATP Standard (10 mM) in 90 µL of ddH_2O to make an ATP Standard (1 mM).

***Note:** Aliquot all reagents except for the ATP Assay Buffer to minimize freezing and thawing.

3. Sample preparation

A. For cell samples

1) Harvest cells (initial recommendation = 1 x 10^6 cells) and wash once with PBS. Then resuspend in 100 µL ATP Assay Buffer.

2) Homogenize cells by pipetting up and down a few times.

3) Centrifuge at 13000 x g at 4°C for 5 min.

4) Transfer supernatant to a new tube and keep on ice.

5) Cell samples may contain enzymes that interfere with the assay. Remove these enzymes by following step 4 to deproteinize.

B. For tissue sample

1) Weigh tissue (initial recommendation = 10 mg) and wash it once with PBS.

2) Homogenize 10 mg tissue in 100 µL of ATP Assay Buffer on ice.

3) Centrifuge at 4°C, 13000 x g for 2-5 min.

4) Transfer supernatant to a new tube and keep on ice.

5) Tissue samples may contain enzymes that interfere with the assay. Remove these enzymes by following step 4 to deproteinize.

C. For plasma, serum, and other liquid samples

1) Collect plasma or serum with heparin instead of EDTA or other chelators.

2) Plasma, serum, and urine samples may contain high levels of protein. Deproteinize samples with a 10 kD spin column and centrifuge at 13000 x g at 4°C for 10 min.

3) Transfer supernatant to a new tube and keep on ice.

D. For red blood cells

Red blood cell samples can be prepared with Red Blood Cell Lysis Buffer following by a deproteinization step. If not using Red Blood Cell Lysis Buffer, red blood cells can be lysed using the following protocol.

1) Collect 1 x 10^7 red blood cells and add 100 µL of ATP Assay Buffer for homogenization on ice.

2) Lyse cells by snap freeze-thaw cycles.

3) Centrifuge at 4°C, 13000 x g for 2 min.

4) Transfer supernatant to a new tube and keep on ice.

5) Samples may contain enzymes that interfere with the assay. Remove these enzymes by following step 4 to deproteinize.

4. PCA deproteinization

1) Add ice cold 4 M PCA to a final concentration of 1 M in the sample supernatant and briefly vortex to mix well.

***Note:** Samples with high protein concentrations may require more PCA.

2) Incubate on ice for 5 min.

***Note:** Do not extend incubation time, as longer incubation may result in loss of ATP.

3) Centrifuge at 4°C, 13000 x g for 2 min.

4) Transfer supernatant to a new tube and keep on ice.

5) Add ice cold 2 M KOH equivalent to 20-35% volume of the supernatant and vortex briefly to mix well. This step can precipitate excess PCA and neutralize the sample.

6) After neutralization, test 1 µL of sample with pH paper, and keep pH in the range of 6.5-8.0. If necessary, adjust pH with 0.1 M KOH or PCA.

7) Centrifuge at 4°C, 13000 x g for 15 min.

8) Transfer supernatant to a new tube and keep on ice. The samples are now ready for assay.

9) To calculate the dilution factor DDF in the deproteinization step, apply the following formula

$$DDF=\frac{\text{initial sample volume + volume PCA + volume KOH}}{\text{initial sample volume}}$$

5. Standard preparation

1) For colorimetric assay: transfer 0, 2, 4, 6, 8, and 10 µL of ATP Standard (1 mM) into a series of wells and adjust with ATP Assay Buffer to 50 µL/well to generate 0, 2, 4, 6, 8, and 10 nmol standards per well.

2) For fluorescence assay: dilute ATP Standard (1 mM) to 100 µM with ddH_2O. Transfer 0, 2, 4, 6, 8, and 10 µL of ATP Standard (100 µM) to a series of wells and adjust with ATP Assay Buffer to 50 µL/well to

generate 0, 0.2, 0.4, 0.6, 0.8, and 1 nmol standards per well.

***Note:**

a) Fluorescence detection is preferred for small-volume samples.

b) Use black 96-well plates for fluorescence assays.

6. Sample assay

1) Detection working solution preparation: Prepare a fresh working solution according to the table below. Each well requires 50 µL. Select the appropriate formulation based on the detection method (colorimetric or fluorescence).

2) (Optional) Background control working solution preparation: Endogenous compounds in the sample may interfere with the assay. It is recommended to set up a background control group.

Components	Colorimetric detection	
	Detection working solution	Background control working solution (optional)
ATP Assay Buffer	44 µL	46 µL
Amplex Red	2 µL	2 µL
Enzyme Solution	2 µL	-
Cofactor	2 µL	2 µL
Total volume	50 µL	50 µL

Components	Fluorescence detection	
	Detection working solution	Background control working solution (optional)
ATP Assay Buffer	45.8 µL	47.8 µL
Amplex Red	0.2 µL	0.2 µL
Enzyme Solution	2 µL	-
Cofactor	2 µL	2 µL
Total volume	50 µL	50 µL

3) Set up reaction wells as follows.

Standards	50 µL standard at different concentrations
Samples	Add 1-50 µL of sample, adjust the volume to 50 µL/well with Assay Buffer

Background control (optional)	Add 1-50 μL of sample, adjust the volume to 50 μL/well with Assay Buffer

***Note:** If needed, pre-dilute the sample to ensure readings fall within the standard curve range.

4) For standards, and sample wells, add 50 μL of the detection working solution to each well. For background control wells, add 50 μL of background control working solution to each well. Mix the 96-well plate well and incubate at room temperature for 30 min.

***Note:** The optimal incubation time can be adjusted according to the specific experiment.

5) Colorimetric detection: Measure absorbance at 570 nm.

Fluorescence detection: Measure fluorescence intensity (Ex/Em = 535/587 nm).

7. The result is calculated

1) Subtract the blank (standard concentration = 0) reading from all readings. If a background control was used, also subtract the background control reading from the corresponding sample reading.

2) Generate a standard curve and interpolate the corrected sample signal to obtain ATP amount (B).

3) ATP concentration in the sample is calculated as:

ATP Concentration (nmol/μL or μmol/mL or mM) = B÷V×d×DDF

B is the ATP amount (nmol or mM) in the sample calculated from standard curve

V is the sample volume added to the reaction system (μL)

D is the sample dilution factor

DDF is the deproteinization dilution factor

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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