ATP ColorimetricFluorometric Assay Kit

(catalog #107002; 100 assays; store at -20°C)

Description:

Adenosine-5'-triphosphate (ATP) is a multifunctional nucleotide used in cells as a coenzyme. ATP is the main energy source for the majority of cellular functions. ATP also is critically involved in maintaining cell structure by facilitating assembly and disassembly of elements of the cytoskeleton. ATP Colorimetric and Fluorometric Assay kit is designed to be a robust, simple method which utilizes the phosphorylation of glycerol to generate a product that is easily quantified by colorimetric(OD = 570 nm) or fluorometric (Ex/Em = 535/590 nm) methods. The assay can detect down to 0.5 µM of ATP in various samples.

APPLICATIONS

Direct Assays: as low as 0.5 µM of ATP in cells and other biological samples. Assay of enzymes that produce or degrade ATP.

KEY FEATURES

Sensitive and accurate: Use 10 µL samples. Detection range 0.5-1000µM in 96-well plate assay.
Simple and high-throughput: Simple procedure; takes less than 30 minutes. Kit is designed to be a robust method.

Kit Contents for 100 Assays:

1. Assay buffer 24 mL
2. Probe 120 µL
3. Substrate 120 µL
4. Enzyme 240 µL
5. ATP standard 100µL 50mM

Storage:

Store kit at -20°C. Shelf life of three months. Except Enzyme warm all of the component to room temperature before use. Briefly centrifuge all small vials prior to opening.

Protocol:

1. Standard Curve Preparations:

For the colorimetric assay, dilute 2 µL of the ATP Standard with 98 µL of ddH2O to generate 1 mM ATP standard. Add 0, 3, 6 and 10 µL into a Clear flat-bottom 96-well plate and adjust volume to

10 µL/well with assay buffer to generate 0, 0.3, 0.6 and 1 mM of ATP Standard. For the fluorometric assay (Detection sensitivity is 10-100 fold higher with the fluorometric than with the Colorimetric assay), further dilute the ATP Standard to 0- 50 µM with the ddH_2O; transfer 10 µL series dilute ATP std into a blank 96-well plate.

2. Sample Preparation:

Tissue (1-10 mg) or cells (1 x 10^6) can be lyses in 100 µL of Assay Buffer. Due to the liability of ATP, for more accurate assays, the sample should be quick frozen using liquid N2 or dry ice if it is to be assayed at a later date. Centrifuge ice cold at 15,000xg for 2 minutes to pellet insoluble materials. Collect supernatant and add 10 µL to 96-well plate.

3. ATP Reaction Mix: Prepare enough mix for each well by mixing 90 µL assay buffer, 1 µL substrate, 1µL probe, 2 µL enzyme for the number of samples and standards. Mix well. Add 90 µL of the Reaction Mix to each well containing the ATP Standard and test samples.

4. Tap plate lightly to mix. Incubate at room temperature for 20 minutes, protect from light.

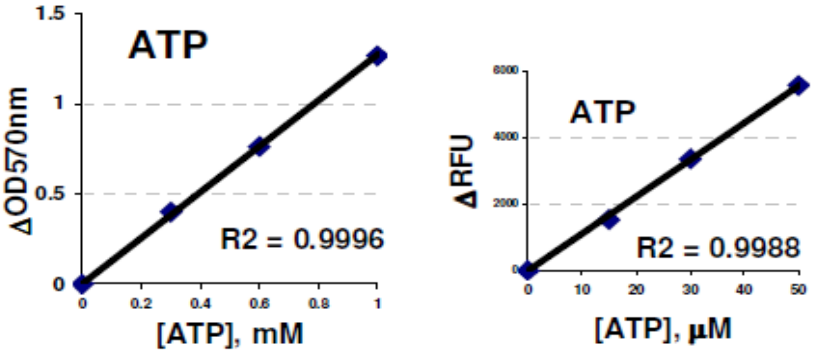
5. Measure OD at 570 nm for colorimetric assay or Ex/Em = 530/590 nm for fluorometric assay.

6. Calculation: Correct background by subtracting the value of the 0 ATP standard (blank) from all standard readings. Plot the value against standard concentration. Determine the slope using linear regression fitting.

$ATP = (OD_{sample}-OD_{blank})/Slope\ (mM)$ Or $ATP = (RFU_{sample}-RFU_{blank})/Slope\ (\mu M)$

Where: OD_{SAMPLE} and OD_{blank} are optical density values of the sample and buffer; RFU_{SAMPLE} and RFU_{blank} are optical fluorescence values of the sample and buffer.

If unknown sample results over standard curve range, dilute sample in assay buffer. Repeat the assay; multiply the results by the dilution factor n.



Standard Curve in 96-well plate.

Note: This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals. Avoid contact with eye,skin and clothing. Do not ingest. Wear gloves.

RELATED PRODUCTS:

ADP Colorimetric/Fluorometric Assay Kit (catalog# 107004)
ADP/ATP Ratio Assay Kit(Bioluminescent) (catalog# 107003)