ADP Colorimetric/Fluorometric Assay Kit

(catalog #107004; 100 assays; store at -20°C)

Description:

ADP is a product of ATP dephosphorylation and it can be rephosphorylated to ATP. ADP levels regulate several enzymes involved in intermediary metabolism. ADP conversion to ATP primarily occurs within the mitochondrion and chloroplast although several such processes occur in the cytoplasm. Genorise's ADP Colorimetric and Fluorometric Assay kit is designed to be a robust, simple method in which ADP is converted to ATP and pyruvate. The generated pyruvate can be quantified by colorimetric (Absorbance = 570 nm) or fluorometric method (Ex/Em 530/590 nm). The assay is simple, sensitive, stable and high-throughput adaptable. The assay can detect as low as 1 µM ADP in various biological samples.

APPLICATIONS

Direct Assays: as low as 1 µM of ADP in cells and other biological samples.

KEY FEATURES

Sensitive and accurate: Use 10 µL samples. Detection range 1-1000µM 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 12 mL
2. Substrate 120 µL
3. Probe 120 µL
4. ADP standard 100µL 50mM
5. Enzyme 600µL

Storage:

Store kit at -20℃. 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 ADP Standard with 98 µL of ddH_2O to generate 1 mM ADP 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 ADP Standard.

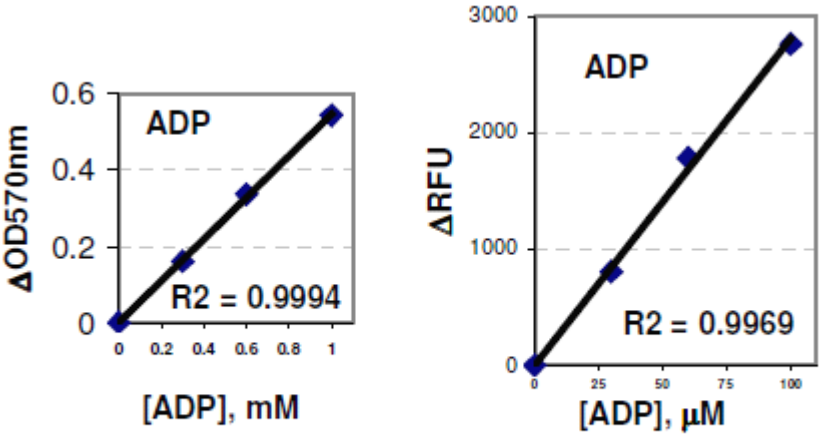
For the fluorometric assay (Detection sensitivity is 10-100 fold higher with the fluorometric than with the colorimetric assay), further dilute the ADP Standard to 1- 100 µM with the ddH_2O; transfer 10 µL series dilute ADP std into a blank 96-well plate.

2. Sample Preparation
   Tissue (1-10 mg) or cells (1 x 10^6) can be lyses in 100 µL of Assay Buffer. 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. ADP Reaction Mix:
   Prepare enough mix for each well by mixing 85 µL assay buffer, 1 µL substrate, 1 µL probe, 5 µL enzyme for the number of samples and standards. Mix well. Add 90 µL of the Reaction Mix to each well containing the ADP 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 ADP standard (blank) from all standard readings. Plot the value against standard concentration. Determine the slope using linear regression fitting.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.

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

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.

LITERATURE

1. Jeffrey A. Dietrich,Adrienne E. McKee, and Jay D. Keasling(2010). High-Throughput Metabolic Engineering: Advances in Small-Molecule Screening and Selection. Annual Review of Biochemistry 79: 563-590

2. A. M. REYNARD, L. F. HASS, D. D. JACOBSEN, AND P. D. BOYER (1961). The Correlation of Reaction Kinetics and Substrate Binding with the Mechanism of Pyruvate kinase JBC 236(8):2277-2283

RELATED PRODUCTS:

ATP Colorimetric/Fluorometric Assay Kit (catalog# 107002)
ADP/ATP Ratio Assay Kit(Bioluminescent) (catalog# 107003)