Tribioscience

ADP Colorimetric/Fluorometric Assay Kit (TBS2020; 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.

Tribioscience'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 1-1000 µM in a 96-well plate assay.
Simple: Just load and incubate procedure; takes less than 60 minutes.
High-throughput: Kit is designed to be a robust method for High-throughput manner.

KIT CONTENTS

Name	Size (100 tests)
ADP Assay Buffer 24 mL	24 mL
Probe	120 µL
Substrate	120 µL
Enzyme Mix	600 µL
ADP standard 50 mM	100 µL

STORAGE AND HANDLING

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

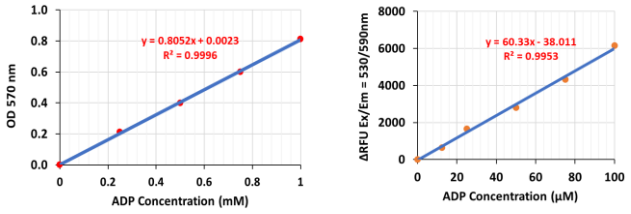
ASSAY PROTOCOL

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, 2.5, 5, 7.5 and 10 µL into a Clear flat-bottom 96-well plate and adjust volume to 10 µL/well with assay buffer to generate 0, 0.25, 0.5, 0.75 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, black 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 83 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. Tap plate lightly to mix.
4. Incubation: Incubate at 37ºC for 30 minutes, protect from light.
5. Measurement: 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.

ADP = (OD_{sample}-OD_{blank})/Slope (mM)
Or
ADP = (RFU_{sample}-RFU_{blank})/Slope (µM)
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. **(Note:** If unknown sample results over standard curve range, dilute sample with assay buffer. Repeat the assay; multiply the results by the dilution factor *n*.



Standard Curve in 96-well plate.

RELATED PRODUCTS:

LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay (TBS2010)
Cell Count Kit -8 (TBS2022)
XTT Cell Viability Assay (TBS2021)
Caspase-3 Colorimetric Assay (TBS2030)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102)
Cytochrome c Reductase Activity Assay (TBS2116)

Research use only