Product Name: ADP Colorimetric/Fluorometric Assay Kit

Cat. #: T2020

Size: 100 tests

Ship at 4℃, Store at -20℃ (Except Enzyme, warm all of the components to room temperature before use. Briefly centrifuge all small vials prior to opening.)

Shelf Life: 3 months

Description: ADP is a product of ATP dephosphorylation, it can be rephosphorylated to ATP, and regulates several enzymes involved in intermediary metabolism. ADP conversion to ATP primarily occurs within the mitochondria and chloroplast although several such processes occur in the cytoplasm. This 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 with the capability to detect as low as 1 µM ADP in various biological samples.

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

Features

Sensitive and Accurate: Use 10 L samples. Detection range 1-1000µM in 96-well plate assay.

Simple and High-Throughput: Simple procedure; less than 30 minutes. Kit is designed to be a robust method.

Kit Contents

Assay Buffer	24mL		Enzyme	600µ
Probe	120µL		ADP Standard (50mM)	100µ
Substrate	120µL			

Assay Protocol

1. Standard Curve Preparations:

For the colorimetric assay, dilute 2 L of the ADP Standard with 98 L of ddH2O to generate a 1 mM ADP standard. Add 0, 3, 6 and 10 L into a Clear flat-bottom 96-well plate and adjust volume to 10 L/well with assay buffer to generate 0, 0.3, 0.6 and 1 mM of ADP Standard. For the fluorometric assay (Detection sensitivity is 10-100x higher with the fluorometric than with the colorimetric assay), further dilute the ADP Standard to 1-100 M with the ddH2O; transfer 10 L series dilute ADP std into a blank 96-well plate.

2. Sample Preparation:

Tissue (1-10 mg) or cells (1 x 106) 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 later. Centrifuge ice cold at 15,000xg for 2 minutes to pellet insoluble materials. Collect supernatant and add 10 L to 96-well plate.

3. ADP Reaction Mix: Prepare enough mix for each well by mixing 85 L assay buffer, 1 L substrate, 1 L probe, 5 L 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.

ADP = (ODsample-ODblank)/Slope (mM) Or

ADP = (RFUsample-RFUblank)/Slope (µM)

Where: ODSAMPLE and ODblank are optical density values of the sample and buffer; RFUSAMPLE and RFUblank 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.

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