ADP Assay Kit (Fluorometric)

(Catalog #BN00488; 100 assays; Store at -20°C)

I. Introduction:

Adenosine diphosphate (ADP) is an organic molecule that plays a critical role in cellular metabolism and energy transfer reactions. ADP is a product of ATP dephosphorylation and it can be rephosphorylated to ATP. These dephosphorylations and rephosphorylations occur via various phosphorylases and kinases. ADP is stored in platelets and can be released to interact with variety of purinergic receptors. ADP levels regulate several enzymes involved in intermediary metabolism. ADP conversion to ATP primarily occurs within the mitochondrion and chloroplast although several such processes also occur in the cytoplasm. Conventionally, ADP levels are measured by luciferase/luciferin mediated assays after ADP is converted to ATP. However, the luciferase system is unstable and luminescence equipment is generally not available in most laboratories. Assay Genie's ADP Assay Kit provides a sensitive fluorescence method to detect ADP. The kit is suitable for measuring ADP levels in various samples, including the ones that contain reducing substances, which may interfere with oxidase-based assays. In this assay, ADP, in the presence of ADP Enzyme Mix is converted to an intermediate, which reacts with the fluorescent probe to generate a strong stable signal at Ex/Em = 535/587 nm. Assay Genie's ADP Assay kit provides an ultrasensitive, fast and high-throughput ready assay for detection of as low as 10 pmol or 0.05 µM of ADP in samples.

II. Application:

- Measurement of ADP in various tissues/cells
- Analysis of metabolism and cell signaling in various cell types

III. Sample Type:

- Animal tissues - Liver, Muscle, Heart etc.
- Cell culture - Adherent or suspension cells

IV. Kit Contents:

Components	BN00488	Cap Code	Part Number
ADP Assay Buffer	25 ml	WM	BN00488-1
ADP Enzyme Mix I	1 Vial	Purple	BN00488-2
ADP Enzyme Mix II	1 Vial	Red	BN00488-3
ADP Developer	1 Vial	Green	BN00488-4
ADP Probe (in DMSO)	0.4 ml	Blue	BN00488-5
ADP Standard	1 Vial	Yellow	BN00488-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom.
- Multi-well spectrophotometer (fluorescent plate reader)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Once opened, store the kit components as per the respective temperatures mentioned below. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **ADP Assay Buffer:** Bring to room temperature (RT) before use. Store at 4°C or -20°C.
- **ADP Enzyme Mix I, ADP Enzyme Mix II & ADP Developer:** Reconstitute each vial with 220 µl ADP Assay Buffer respectively. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze thaw. Keep on ice while in use. Stable for 2 months at -20°C.
- **ADP Probe:** Thaw the DMSO solution at room temperature before use. Aliquot and store at -20°C. Use within two months.
- **ADP Standard:** Reconstitute with 1 ml dH_2O to generate 1 mM ADP stock solution. Aliquot and store at –20°C. Dilute the 1 mM stock solution 1:100 with dH_2O to make 10 µM (10 pmol/µl) working solution just before use. Make only as much as needed. Keep on ice while in use.

VII. ADP Assay Protocol:

1. Sample Preparation: Rapidly homogenize fresh tissue (2 mg) or cells (2 x 10^5) with 200 µl ice cold ADP Assay Buffer for 10 minutes on ice. Centrifuge at 10,000 g for 5 min at 4ºC. Collect the supernatant. Add 1-50 µl sample (1-50 µg) into a 96 well plate. Adjust the final volume to 50 µl with the ADP Assay Buffer.

Notes:

a.) For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
b.) For samples having high background, prepare a parallel sample well as the background control.
c.) Enzymes in some samples may interfere with the assay. Enzymes can be removed by 10 kD spin column.

2. Standard Curve Preparation: Add 0, 2, 4, 6, 8 and 10 µl of 10 µM ADP Standard into a series of wells in a 96 well plate to generate 0, 20, 40, 60, 80 and 100 pmol/well of ADP Standard. Adjust final volume to 50 µl/well with ADP Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 80 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
ADP Enzyme Mix I	2 µl	---
ADP Enzyme Mix II	2 µl	2 µl
ADP Developer	2 µl	2 µl
Assay Kit	4 µl	4 µl
ADP Assay Buffer	40 µl	42 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples. Mix well.

Note: For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. Measurement: Incubate for 30 min at RT. Measure fluorescence (Ex/Em = 535/587 nm).

5. Calculation: Subtract the 0 Standard reading from all readings. Plot the ADP Standard Curve. Note: For samples having high background, correct sample background by subtracting the value derived from the background control from all sample readings. Apply the corrected sample reading to the Standard Curve to get B pmol of ADP amount in the sample.

Sample ADP concentration = B/V x Dilution Factor = pmol/µl = µM

Where:
B is the ADP amount from the Standard Curve (pmol).
V is the sample volume added into the reaction well (µl).
ADP molecular weight: 501.32 g/mole.

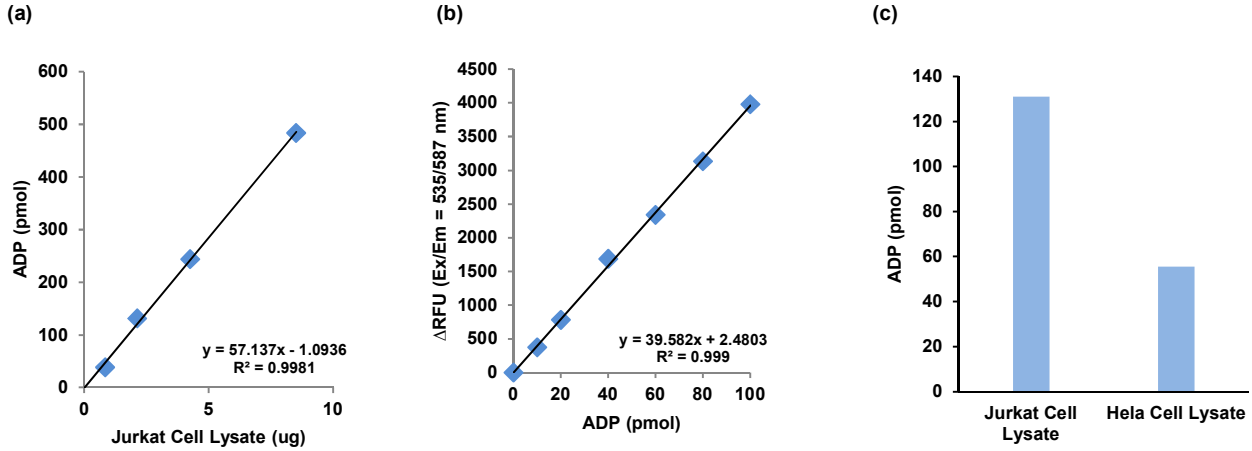

Figure: (a) ADP Standard Curve, (b) Measurement of ADP in 1-10 µg Jurkat cell lysate, (c) Measurement of ADP in 2 µg HeLa & Jurkat cell lysate respectively. Assays were performed following the kit protocol.

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