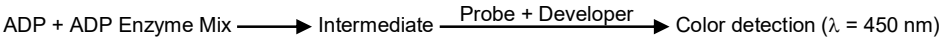
ADP Colorimetric Assay Kit II

(Catalog # BN00619; 100 assays; Store at -20°C)

I. Introduction:

ADP is a product of ATP dephosphorylation and it can be rephosphorylated to ATP. Dephosphorylation and rephosphorylation 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 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 not generally available in most laboratories. Assay Genie's ADP Assay kit II is suitable for measuring ADP levels in samples 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 reduces a colorless Probe to a colored product with strong absorbance at 450 nm. ADP Assay Kit II is simple, fast and high-throughput ready. It can detect less than 20 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	BN00619	Cap Code	Part Number
ADP Assay Buffer	27 ml	WM	BN00619-1
ADP Enzyme Mix (Lyophilized)	1 vial	Purple	BN00619-2
Developer (Lyophilized)	1 vial	Green	BN00619-3
Probe (Lyophilized)	1 vial	Red	BN00619-4
ADP Standard (Lyophilized)	1 vial	Yellow	BN00619-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **ADP Enzyme Mix**: Reconstitute with 220 μl ADP Assay Buffer. 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.
- **Developer**: Reconstitute with 220 μl ADP Assay Buffer. 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.
- **Probe**: Reconstitute with 220 μl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- **ADP Standard:** Reconstitute with 100 μl dH_2O to generate 10 mM (10 nmol/μl) ADP Standard solution. Aliquot and Store at –20°C. Keep on ice while in use. Use within two months.

VIII. ADP Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (20 mg) or cells (2 x 10^6) with 200 µl ice cold ADP Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 μl sample (50-200 μg) into a 96 well plate. Adjust final volume to 50 µl with 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 NADH, prepare a parallel sample well as the background control to subtract interference from NADH.
   c.) Enzymes in some samples may interfere with the assay. Enzymes can be removed by 10 kD spin column.
2. **Standard Curve Preparation:** Dilute ADP Standard to 1 mM by adding 10 μl of 10 mM ADP Standard to 90 μl dH_2O. Mix well. Add 0, 2, 4, 6, 8 and 10 μl of 1 mM ADP Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8 and 10 nmol/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 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
ADP Assay Buffer	44 μl	46 µl
ADP Enzyme Mix	2 µl	----

Developer	2 µl	2 µl
Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples. Mix well.
Note: For samples having high NADH, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement:** Incubate for 20 min at 37^{0}C and measure OD_{450nm}.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the ADP Standard Curve. **Note:** For samples having high NADH, 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 nmol of ADP amount in the sample.

Sample ADP concentration = B/V x Dilution Factor = nmol/ml = μM

Where: **B** is the ADP amount from the Standard Curve (nmol).
V is the sample volume added into the reaction well (ml).

ADP molecular weight: 501.32 g/mole.

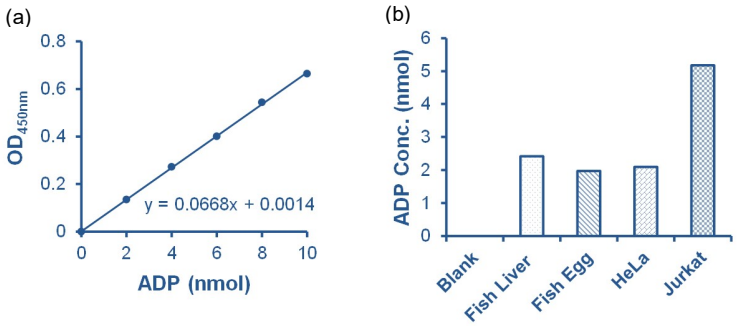


Figure 1. ADP Standard Curve [a]. Measurement of ADP in fish liver (100 μg), fish egg (200 μg) & HeLa & Jurkat cell lysate (100 μg) [b]. Assays were performed following kit protocol.

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