ADP Colorimetric/Fluorometric Assay Kit

(Catalog# BN00618; 100 Assays; Store at -20°C)

I. Introduction:

ADP is a product of ATP dephosphorylating and it can be rephosphorylated to ATP. De-phosphorylation and rephosphorylation occur via various phosphatases, phosphorylases and kinases. ADP is stored in platelets and can be released to interact with a 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 newly designed ADP Assay Kit provides a convenient colorimetric and fluorometric means to measure ADP level. In the assay, ADP is converted to ATP and pyruvate. The generated pyruvate can be quantified by colorimetric (λ_{max} = 570 nm) or fluorometric methods (Ex/Em 535/587 nm). The assay is simple, sensitive, stable and high-throughput adaptable. The assay can detect as low as 1 µM ADP in biological samples.

II. Kit Contents:

Components	BN00618	Cap Code	Part Number
ADP Assay Buffer	25 ml	WM	BN00618-1
ADP Probe (in DMSO)	0.2 ml	Red	BN00618-2A
ADP Converter	1 vial	Purple	BN00618-3
ADP Developer Mix	1 vial	Green	BN00618-4
ADP Standard (1 µmole lyophilized)	1 vial	Yellow	BN00618-5

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm ADP Assay Buffer to room temperature prior to use. Briefly centrifuge all vials prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Preparation and Storage Conditions:

ADP Probe: Ready to use as supplied. Warm up >18°C to melt frozen DMSO before use. Mix well, store at –20°C, protect from light and moisture. Use within two months.

ADP Converter and ADP Developer Mix: Dissolve with 220 µl ADP Assay Buffer separately. Pipette up and down to dissolve. Store at –20°C. Use within two months.

ADP Standard: Dissolve in 100 µl dH_2O to generate 10 mM stock solution. Keep cold while in use. Store at -20°C.

V. ADP Assay Protocol:

1. **Standard Curve Preparations:**

   For the Colorimetric Assay: Dilute the ADP Standard to 1 nmol/ µl by adding 10 µl of the 10 mM Standard to 90 µl of ADP Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with ADP Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of ADP Standard.

   For the Fluorometric Assay: Dilute the ADP Standard to 0.1 nmol/ µl (the fluorometric assay is 10 to 100 fold more sensitive than the colorimetric assay). Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of ADP standard.

2. **Sample Preparation:** Liquid samples can be measured directly. Tissue (10 mg) or cells (10^6) can be homogenized in 100 µl of ADP Assay Buffer, spin at 12,000 X g for 5 min to remove insoluble materials. Add 1-50 µl sample to each well in a 96-well plate; bring the volume to 50 µl with Assay Buffer.

3. Intracellular ADP level is usually in the range of 0.1-3 mM. We suggest testing several doses of your sample to ensure the readings are within the standard curve range.

4. **ADP Reaction Mix:** Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

	Colorimetric Assay	Fluorometric Assay
ADP Assay Buffer	44 µl	45.8 µl
ADP Probe	2 µl	0.2 µl*
ADP Converter**	2 µl	2 µl
ADP Developer	2 µl	2 µl

Notes:

*For the fluorometric assay, use 1/10 of ADP probe to reduce fluorescence background.

**Pyruvate generates background. If significant amount of pyruvate is suspected in your samples, a sample pyruvate background control need to be performed by replacing the ADP Converter with 2 µl of assay buffer. Then follow the same protocol as the sample. In the absence of ADP converter, the assay detects only pyruvate, not ADP. The pyruvate background reading can be subtracted from the ADP readings.

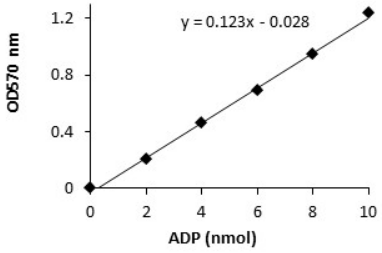
5. Add 50 µl of the Reaction Mix to each well containing the ADP Standard and test samples. Incubate at room temperature for 30 minutes, protect from light.

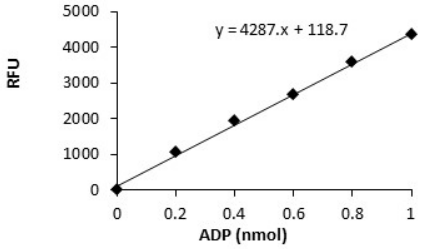
6. Measure O.D. 570 nm for colorimetric assay or Ex/Em 535/587 nm for fluorometric assay.

7. **Calculation:** Subtracting the 0 ADP control from all standard and sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot ADP standard curve. Apply the sample readings to the standard curve to get ADP amount in the sample wells. The ADP concentrations in the test samples:

   C = Ay/Sv (nmol/ µl, µmol/ml or mM)

   Where: Ay is the ADP amount (nmol) in your sample from the standard curve.
   Sv is the sample volume (µl) added to the assay well.
   ADP molecular weight: 427.2.





FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		