

ADP Colorimetric/Fluorometric Assay Kit

(Catalog# K355-100; 100 Assays; Store at -20°C)

I. Introduction:

ADP is a product of ATP dephosphorylating and it can be rephosphorylated to ATP. Dephosphorylation 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. BioVision'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 (OD = 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 μ M ADP in biological samples.

II. Kit Contents:

Components	K355-100	Cap Code	Part Number
ADP Assay Buffer	25 ml	WM	K355-100-1
ADP Probe (in DMSO)	0.2 ml	Red	K355-100-2A
ADP Converter	1 vial	Purple	K355-100-4
ADP Developer Mix	1 vial	Green	K355-100-5
ADP Standard (1 μ mol lyophilized)	1 vial	Yellow	K355-100-6

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 to 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 μ l ADP Assay Buffer separately. Pipette up and down to dissolve. Store at -20°C. Use within two months.

ADP Standard: Dissolve in 100 μ l dH₂O 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/ μ l by adding 10 μ l of the 10 mM Standard to 90 μ l of ADP Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 μ l into a series of standards wells. Adjust volume to 50 μ l/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/ μ l (the fluorometric assay is 10 to 100 fold more sensitive than the colorimetric assay). Add 0, 2, 4, 6, 8, 10 μ l into a series of standards wells. Adjust volume to 50 μ l/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 (10⁶) can be homogenized in 100 μ l of ADP Assay Buffer, spin at 12,000 X g for 5 min to remove insoluble materials. Add 1-50 μ l sample to each well in a 96-well plate; bring the volume to 50 μ l with Assay Buffer.

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

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

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 μ l 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.

- Add 50 μ l of the Reaction Mix to each well containing the ADP Standard and test samples. Incubate at room temperature for 30 minutes, protect from light.
- Measure OD at 570 nm for colorimetric assay or Ex/Em 535/587 nm for fluorometric assay.
- Calculation:** Subtract the 0 ADP control reading 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 can be calculated as follows:

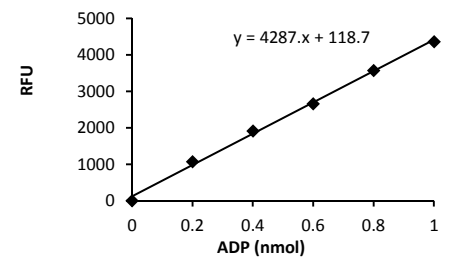
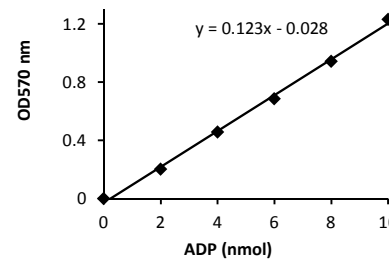
$$C = Ay/Sv \text{ (nmol/ } \mu\text{l, } \mu\text{mol/ml or mM)}$$

Where:

Ay is the ADP amount (nmol) in your sample from the standard curve.

Sv is the sample volume (μ l) added to the assay well.

ADP molecular weight: 427.2.



VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
 Glucose, Sucrose, Glycogen Assay Kit
 Glutathione Assay Kit
 NAD/NADH and NADP/NADPH Assay Kits
 ATP Fluorometric/Colorimetric Assay Kit

Cell Proliferation & Senescence Kits
 Cholesterol, LDL/HDL Assay Kits
 Ethanol and Uric Acid Assay Kit
 Glucose and Lactate Assay Kits
 Pyruvate Assay Kit

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.