

## ADP Colorimetric/Fluorometric Assay Kit

(Catalog# K355-100; 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 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 an 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 ( $\lambda_{\max} = 570 \text{ nm}$ ) or fluorometric method (Ex/Em 535/587 nm). The assay is simple, sensitive, stable and high-throughput adaptable. The assay can detect as low as  $1 \mu\text{M}$  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 (lyophilized)	1 vial	Red	K355-100-2
DMSO (anhydrous)	0.5 ml	Brown	K355-100-3
ADP Converter	1 vial	Purple	K355-100-4
ADP Developer Mix	1 vial	Green	K355-100-5
ADP Standard (1 $\mu\text{mole}$ lyophilized)	1 vial	Yellow	K355-100-6

### III. Storage and Handling:

Store kit at  $-20^\circ\text{C}$ , protect from light. Warm ADP Assay Buffer to room temperature prior to use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

### IV. Reagent Preparation and Storage Conditions:

**ADP Probe:** Dissolve with 220  $\mu\text{l}$  of DMSO (provided, warm up  $>18^\circ\text{C}$  to become liquid) before use. Mix well, store at  $-20^\circ\text{C}$ , protect from light and moisture. Use within two months.

**ADP Converter and ADP Developer Mix:** Dissolve with 220  $\mu\text{l}$  ADP Assay Buffer separately. Pipette up and down to dissolve. Store at  $-20^\circ\text{C}$ . Use within two months.

**ADP Standard:** Dissolve in 100  $\mu\text{l}$  dH<sub>2</sub>O to generate 10 mM stock solution. Keep cold while in use. Store at  $-20^\circ\text{C}$ .

### V. ADP Assay Protocol:

#### 1. Standard Curve Preparations:

**For the Colorimetric Assay:** Dilute the ADP Standard to 1 nmol/ $\mu\text{l}$  by adding 10  $\mu\text{l}$  of the 10 mM Standard to 90  $\mu\text{l}$  of ADP Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  into a series of standards wells. Adjust volume to 50  $\mu\text{l}$ /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/ $\mu\text{l}$  (the fluorometric assay is 10 to 100 fold more sensitive than the colorimetric assay). Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  into a series of standards wells. Adjust volume to 50  $\mu\text{l}$ /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 ( $10^6$ ) can be homogenized in 100  $\mu\text{l}$  of ADP Assay Buffer, spin at 12,000 X g for 5 min to remove insoluble materials. Add 1-50  $\mu\text{l}$  sample to each well in a 96-well plate, bring the volume to 50  $\mu\text{l}$  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.

3. **ADP Reaction Mix:** Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50  $\mu\text{l}$  Reaction Mix containing:

	Colorimetric Assay	Fluorometric Assay
ADP Assay Buffer	44 $\mu\text{l}$	45.8 $\mu\text{l}$
ADP Probe	2 $\mu\text{l}$	0.2 $\mu\text{l}$ *
ADP Converter**	2 $\mu\text{l}$	2 $\mu\text{l}$
ADP Developer	2 $\mu\text{l}$	2 $\mu\text{l}$

#### 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  $\mu\text{l}$  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.

4. Add 50  $\mu\text{l}$  of the Reaction Mix to each well containing the ADP Standard and test samples. Incubate at room temperature for 30 minutes, protect from light.

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

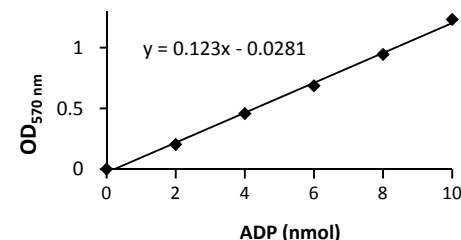
6. **Calculation:** Subtracting the 0 ADP control from all Standard and sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot ADP standard curve. Apply the sample readings to the standard curve to get ADP amount in the sample wells. The ADP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

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

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

Sv is the sample volume ( $\mu\text{l}$ ) added to the assay well.

ADP molecular weight: 427.2.



### VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Cell Proliferation & Senescence Kits
Glucose, Sucrose, Glycogen Assay Kit	Cholesterol, LDL/HDL Assay Kits
Glutathione Assay Kit	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit	Glucose and Lactate Assay Kits
ATP Fluorometric/Colorimetric Assay Kit	Pyruvate Assay Kit