Technical Manual

FluoroDazzle ADP Assay Kit

Catalogue Code: BA0063

Pack Size: 100 assays

Research Use Only

DESCRIPTION

Adenosine diphosphate (ADP) is the product of ATP dephosphorylation by ATPases. ADP can be converted back to ATP by ATP synthases. ADP levels regulate several enzymes involved in intermediary metabolism. Conventionally, ADP levels are measured by luciferase/luciferin mediated assays after ADP is converted to ATP. However, since these assays require measurement of ATP in the sample before conversion of ADP to ATP, if the nascent ATP concentration is significantly higher than the ADP concentration, the ATP signal will drown out the ADP signal.

The Assay Genie newly designed FluoroDazzle ADP Assay Kit provides a convenient fluorometric means to measure ADP level even in the presence of ATP. In the assay, ADP is converted to ATP and pyruvate. The generated pyruvate is then quantified by a fluorimetric method ($\lambda_{exc/em}$ = 530/590nm). The assay is simple, sensitive, stable, high-throughput adaptable and can detect as low as 0.1 µM ADP in biological samples.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. As low as 0.1 µM ADP can be quantified.

Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS: Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

ADP determination in cells and other biological samples.

KIT CONTENTS

Reagent A:	6 mL	**Enzyme:**	120 µL	**10% TCA:**	6 mL
Reagent B:	6 mL	**Standard:**	100 µL	**Neutralizer:**	1.5 mL

Storage conditions: store all reagents at -20°C. This product is shipped on ice. Shelf life of at least 6 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

Use black flat-bottom plates. Prior to assay, bring all reagents to room temperature.

Interference: thiols (β-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided.

1. *Standards*. Prepare 900 µL 20 µM ADP Premix by mixing 6 µL 3 mM Standard and 894 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	ADP (µM)
1	50 µL + 0 µL	20
2	30 µL + 20 µL	12
3	15 µL + 35 µL	6
4	0 µL + 50 µL	0

Transfer 40 µL standards into separate wells of the plate.

2. *Sample Preparation*. Samples high in protein and especially those with likely ATPase activity (cell lysate, serum, etc.) need to be deproteinated and neutralized prior to assaying. To deproteinate, add 25 µL 10% TCA per 100 µL sample. Vortex and centrifuge for 10 min at 14000rpm. Transfer 100 µL of clear supernatant to a clean tube and neutralize with 12.5 µL Neutralizer. For cell assays, at least 1×10^5 cells should be used. Cells should be lysed and deproteinated at the same time by homogenization in 100 µL dH_2O plus 25 µL 10% TCA per 2×10^5 cells followed by the centrifugation and

neutralization procedure outlined above. *Note:* Measured ΔRFU's for deproteinated samples need to be multiplied by 1.41 to compensate for the resulting dilution of the sample.

3. Transfer 40 µL of each sample to separate wells of a 96 well plate. For samples containing pyruvate, add 40 µL of each sample to 2 separate wells where one well will serve as the sample blank.
4. Prepare Working Reagent for each well by mixing 45 µL Reagent A, 45 µL Reagent B and 1 µL Enzyme. If the samples contain pyruvate, sample blanks need to be included. For sample blanks, make the following Working Reagent: 45 µL Reagent A + 45 µL Reagent B ***(No Enzyme)***. Add 80 µL of the appropriate Working Reagent to each assay well. Tap plate to mix. Incubate at room temperature for 30 min protected from light.
5. Read fluorescence intensity at λ_{exc} = 530 nm and λ_{em} = 590 nm.

CALCULATION

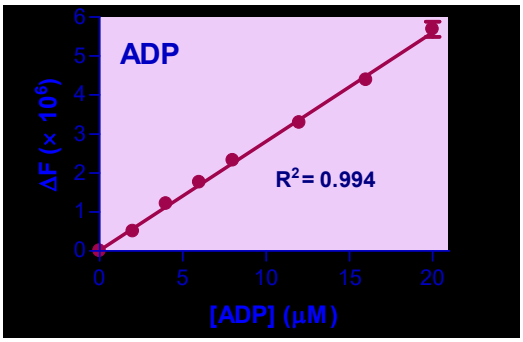
Plot the RFU measured at 30 min for each standard against the standard concentrations. Determine the slope using linear regression fitting. The ADP concentration of a Sample is calculated as

$$[ADP] = \frac{RFU_{SAMPLE} - RFU_{BLANK}}{Slope} \times n \quad (\mu M)$$

where RFU_{SAMPLE} and RFU_{BLANK} are the measured fluorescence values of the sample and sample blank (*or H_2O (std #4) if sample blank not required*) respectively. **Slope** is the slope of the standard curve in μM^{-1}. *n* is the sample dilution factor (1.41 for deproteinated samples). Note: if the Sample ADP concentration is higher than the 20 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black flat-bottom 96-well plates (e.g. VWR cat# 82050-676), centrifuge tubes and plate reader.



ADP Standard Curve in Water

LITERATURE

1. Bradbury DA, et al (2000). Measurement of the ADP:ATP ratio in human leukaemic cell lines can be used as an indicator of cell viability, necrosis and apoptosis. J Immunol Methods. 240:79-92.
2. Chen-Scarabelli C, et al (2004). Turning necrosis into apoptosis: the exacting task that can enhance survival. Am Heart J. 148:196-9.
3. Clement CC (2008). Development of Resorufin Fluorescence Assays for monitoring the ATP-ase Activity of Human Chaperone Hsp-70. *FASEB J.* 22:791.

Contact Details

Dublin, Ireland

Email: info@assaygenie.com

Web: www.assaygenie.com

Technical Support: Techsupport@assaygenie.com