ADP Assay Kit (Fluorometric)

ADP Assay Kit (Fluorometric) is a detection kit for the quantification of ADP in cell culture supernatants.

Catalog number: ARG82131

Package: 100 tests

TABLE OF CONTENTS

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INTRODUCTION

Adenosine diphosphate (ADP), also known as adenosine pyrophosphate (APP), is an important organic compound in metabolism and is essential to the flow of energy in living cells. ADP consists of three important structural components: a sugar backbone attached to adenine and two phosphate groups bonded to the 5 carbon atom of ribose. The diphosphate group of ADP is attached to the 5' carbon of the sugar backbone, while the adenine attaches to the 1' carbon.

ADP can be interconverted to adenosine triphosphate (ATP) and adenosine monophosphate (AMP). ATP contains one more phosphate group than does ADP. AMP contains one fewer phosphate group. Energy transfer used by all living things is a result of dephosphorylation of ATP by enzymes known as ATPases. The cleavage of a phosphate group from ATP results in the coupling of energy to metabolic reactions and a by-product of ADP. ATP is continually reformed from lower-energy species ADP and AMP. The biosynthesis of ATP is achieved throughout processes such as substrate-level phosphorylation, oxidative phosphorylation, and photophosphorylation, all of which facilitate the addition of a phosphate group to ADP. [Provide by Wikipedia: Adenosine diphosphate]

PRINCIPLE OF THE ASSAY

This ADP Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of ADP present in cell culture supernatant. This 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 (exc/em = 530/590nm). The assay is simple, sensitive, stable, high-throughput adaptable and can detect as low as 0.1 µM ADP in cell culture supernatant.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C. Shelf life of 6 months after receipt.

Component	Quantity	Storage information
Reagent A	6 mL	-20°C
Reagent B	6 mL	-20°C
Enzyme	120 µL	-20°C
10% TCA	6 mL	-20°C
Neutralizer	1.5 mL	-20°C
Standard (3 mM)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 590 nm.
- Centrifuge
- Black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Interference: thiols (b-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants: Centrifuge cell culture media at 1,500 x g for 10 minutes at 4°C to remove particulates. Samples should be assayed immediately or stored at-20°C.

Note:

1. Thiols (b-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided.
2. Samples high in protein and especially those with likely ATPase activity (cell lysate, serum, etc.) need to be deproteinated and neutralized prior to assaying.
3. To deproteinate, add 25 µL of 10% TCA per 100 µL sample. Vortex and centrifuge for 10 minutes at 14000 rpm. Transfer 100 µL of clear supernatant to a clean tube and neutralize with 12.5 µL of 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 of distilled water plus 25 µL of 10% TCA per 2×10^5 cells followed by the centrifugation and neutralization procedure outlined above.
4. Measured ΔRFU's for deproteinated samples need to be multiplied by 1.41 to compensate for the resulting dilution of the sample.

REAGENT PREPARATION

- **Working Reagent:** For each well, 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).
- **Standard:** Prepare 900 µL of 20 µM ADP Premix by mixing 6 µL of 3 mM Standard and 894 µL of distilled water. Dilute standard as follows.

Standard tube	Final Standard conc. (µM)	Distilled water	Premix
S1	20	0	50
S2	12	20	30
S3	6	35	15
S4	0	50	0

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice during assays.

1. Add **40 µL** of **Standards** or **sample** into wells of a black 96-well microplate.
2. Add **80 µL** of **Working Reagent** into each well. Tap plate to mix.
   Note: 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).
3. Incubate for **30 minutes** at **room temperature** in the dark.
4. Read the plate with a fluorescence microplate reader using excitation 530 nm filter and emission 590 nm filter.

CALCULATION OF RESULTS

1. 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 below:

   ADP (µM) = [(RFU_{Sample} – RFU_{Blank}) / Slope] x n

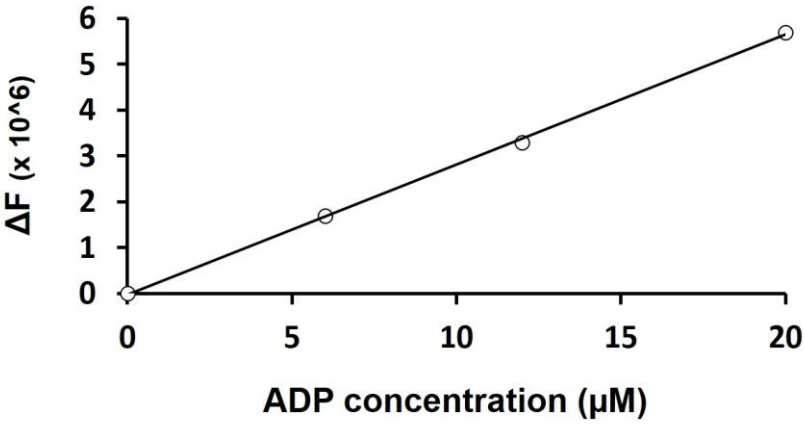
   Note:

   - RFU_{Sample} and RFU_{Blank}: the fluorescence values of the sample and sample blank (or H2O (std #4) if sample blank not required) respectively
   - Slope: the slope of the standard curve in µM-1.
   - n: the sample dilution factor (1.41 for deproteinated samples).

2. 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.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the ADP Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 µM