Technical Bulletin

ADP Assay Kit

Catalogue number MAK518

Product 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.

Our 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 fluorometric method at λex = 530 nm/λem = 590 nm. The assay is simple, sensitive, stable, high-throughput adaptable and can detect as low as 0.1 µM ADP in biological samples.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Reagent A — 6 mL
  Catalogue Number MAK518A
- Reagent B — 6 mL
  Catalogue Number MAK518B
- Enzyme — 120 mL
  Catalogue Number MAK518C
- 10% TCA — 6 mL
  Catalogue Number MAK518D
- Neutralizer — 1.5 mL
  Catalogue Number MAK518E
- Standard (3 mM) — 100 µL
  Catalogue Number MAK518F

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- Black flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Note: Thiols such as β-mercaptoethanol, dithioerythritol, etc., at concentrations >10 µM interfere with this assay and should be avoided.

Procedure

All Samples and Standards should be run in duplicate.

Standard Preparation

1. Prepare 900 µL 20 µM ADP Standard by mixing 6 µL 3 mM Standard and 894 µL purified water.
2. Prepare ADP Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.

Well No.	20 µM Standard (µL)	Purified H2O (µL)	ADP (µM)
1	50	0	20
2	30	20	12
3	15	35	6
4	0	50	0

3. Mix and transfer 40 µL of each Standard into separate wells of a 96-well plate.

Sample Preparation

Note: Samples high in protein and especially those with likely ATPase activity (cell lysate, serum, etc.) need to be deproteinated and neutralized prior to assaying.

Deproteination Instructions

1.Add 25 µL 10% TCA per 100 µL Sample.

2.Vortex and centrifuge for 10 minutes at 14000 rpm.

3.Transfer 100 µL of clear supernatant to a clean tube and neutralize with 12.5 µL Neutralizer.

Cell Samples

1. Assays should be performed with at least 1×10^5 cells.
2. Cells should be lysed and deproteinated at the same time by homogenization in 100 µL purified H_2O plus 25 µL 10% TCA per 2×10^5 cells followed by the centrifugation and neutralization procedure outlined above.
3. Transfer 40 µL of each Sample to separate wells of a 96-well plate.

Note: For samples containing pyruvate, add 40 µL of each Sample to 2 separate wells where one well will serve as the Sample blank.

Working Reagent Preparation

Mix enough reagent for the number of assays to be performed. Prepare working reagent according to Table 2.

Note: If the Samples contain pyruvate, Sample blanks need to be included.

For Sample blanks, prepare Reagent as per Table 2 without Enzyme

Table 2.

Reagent	Working Reagent	Blank Working Reagent
Reagent A	45 µL	45 µL
Reagent B	45 µL	45 µL
Enzyme	1µL	

Transfer 80 µL of the Working Reagent to each assay well. Add 80 µL Blank Working Reagent to the Sample Blank Wells. Tap plate to mix.

Measurement

1. Incubate at room temperature for 30 minutes protected from light.
2. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 590 nm.

Results

Note: Measured ΔRFU's for deproteinated Samples need to be multiplied by 1.41 to compensate for the resulting dilution of the Sample.

1. Subtract the blank value (well #4) from all the standard values.
2. Plot the RFU intensity measured at 30 minutes for each standard against the standard concentrations.
3. Determine the slope using linear regression fitting. The ADP concentration of a Sample is calculated as

$$\text{ADP } (\mu M) = \frac{RFU_{Sample} - RFU_{Blank}}{\text{Slope}} \times DF$$

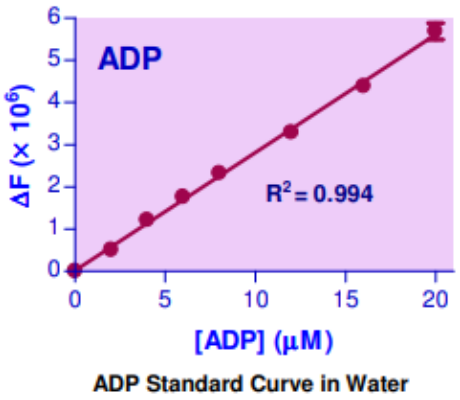
Where,

RFU_{Sample} = Fluorescence intensity readings of the sample
RFU_{Blank} = Fluorescence readings of the Sample blank or purified water
Slope = Slope of the Standard Curve (μM^{-1})
DF = 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, DF.

Figure 1.

Typical Fluorometric ADP Standard Curve in Water



ADP Standard Curve in Water

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