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Product Information

ADP Assay Kit

Catalog Number **MAK133**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Adenosine diphosphate (ADP) is a nucleoside that plays a critical role in energy transfer reactions. ADP is produced from adenosine triphosphate via the action of ATPases. ADP also plays a critical role in platelet function. ADP, stored in plate-dense granules, is released upon platelet activation where it acts on purinergic receptors to mediate intracellular signaling and platelet aggregation.

The ADP Assay kit provides a simple and direct procedure for measuring ADP levels in cells and other biological samples. The assay involves two steps. In the first step, the working reagent lyses cells to release ATP and ADP. In the presences of luciferse, ATP immediately reacts with the substrate (D-luciferin) to produce light. The light intensity is a direct measure of the intracellular ATP concentration and is stable over several minutes.

$$\text{ATP + D-Luciferin + } O_2 \xrightarrow{\text{Luciferase}} \text{oxyluciferin + AMP + } PP_i + CO_2 + \text{light}$$

In the second step, the ADP is converted to ATP through an enzyme reaction. This newly formed ATP then reacts with the D-luciferin as in the first step. The second light intensity measured represents the total ADP and ATP concentration in the sample.

Components

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

Assay Buffer Catalog Number MAK133A	10 mL
Cosubstrate Catalog Number MAK133B	120 µL
ADP Enzyme Catalog Number MAK133C	120 µL
Substrate Catalog Number MAK133D	120 µL
ATP Enzyme Catalog Number MAK133E	120 µL
ADP Standard, 3 mM Catalog Number MAK133F	100 µL

Reagents and Equipment Required but Not Provided.

- Luminometer
- 96 well flat-bottom plate – It is recommended to use white plates with clear bottoms for luminescence assays

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped on dry ice. Storage at –20 °C is recommended.

Procedure

Notes: Assays can be carried out in tubes or in a 96 well plate. The signal of the reaction decreases by ~1% each minute. For best results, it is recommended that the times between the two luminescence measurements be the same for all samples and that the time between adding the Reconstituted Reagent and the luminescence reading is the same for all samples and standards.

Sample Preparation

The assay reagents are compatible with all culture media.

Tissue samples (20 mg) can be homogenized in 200 µL of ice-cold phosphate-buffered saline. Centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material.

Adherent cells (10^3–10^4) can be directly cultured in the assay microplate.

ADP Standards

Mix 5 µL of the 3 mM ADP Standard with 495 µL of water (for cell culture samples, dilute ADP in culture medium) to create a 30 µM working standard. Dilute the 30 µM working standard as indicated in Table 1.

Table 1.
ADP Standards

Number	30 µM ADP Standard	Water	Final ADP Concentration
1	50 µL	0 µL	30 µM
2	40 µL	10 µL	24 µM
3	30 µL	20 µL	18 µM
4	20 µL	30 µL	12 µM
5	15 µL	35 µL	9 µM
6	10 µL	40 µL	6 µM
7	5 µL	45 µL	3 µM
8	0 µL	50 µL	0 µM

Assay Reaction

Bring Assay Buffer, Substrate, and Cosubstrate to room temperature before beginning assay. Thaw ADP enzyme on ice. Aliquot and store unused reagents at –20 °C.

ATP Reaction

1. Transfer 10 µL of standards and 1–10 µL of samples into separate wells of a 96 well plate. For suspension cells, transfer up to 10 µL of the cultured cells (10^3–10^4) into the 96 well plate. Bring samples to a final volume of 10 µL with PBS. For adherent cells cultured in the plate, remove the culture medium and add 90 µL of prepared ATP reagent (see step 2) per well.
   Note: For unknown samples, it is suggested to test several sample dilutions or cell numbers to ensure readings are within the linear range of the standard curve.
2. Prepare ATP reagent according to the scheme in Table 2. Add 90 µL of ATP reagent to each well in plate (samples and standards) and tap plate briefly to mix.

Table 2.
ATP Reagent

Reagent	Volume
Assay Buffer	95 µL
Substrate	1 µL
Cosubstrate	1 µL
ATP Enzyme	1 µL

3. Incubate plate for 10 minutes at room temperature. Read luminescence (relative light units) on a luminometer for the ATP assay (RLU_{ATP}).

ADP Assay

4. Prepare ADP Reagent according to the scheme in Table 3. Add 5 µL of ADP Reagent to each well immediately following the reading of (RLU_{ATP}). Tap plate lightly to mix or by pipetting.

Table 3.
ADP Reagent

Reagent	Volume
Water	5 µL
ADP Enzyme	1 µL

5. Incubate for two minutes at room temperature. Read luminescence for the ADP assay (RLU_{ADP}).

Calculations

Use the values obtained from the appropriate standards to plot a standard curve and determine the slope using linear regression fitting. The amount of ADP present in the samples may be determined from the standard curve using the equations below.
Note: A new standard curve must be set up each time the assay is run.

$$\text{ADP } (\mu M) = \frac{RLU_{ADP} - RLU_{ATP}}{\text{slope}}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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