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Product Information

ADP Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK033**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Adenosine diphosphate (ADP) is a nucleoside diphosphate 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 platelet-dense granules, is released upon platelet activation where it acts on purinergic receptors to mediate intracellular signaling and platelet aggregation.

In this assay, ADP concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the ADP present.

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

ADP Assay Buffer Catalog Number MAK033A	25 mL
ADP Probe, in DMSO Catalog Number MAK033B	0.2 mL
ADP Converter Catalog Number MAK033C	1 vl
ADP Developer Mix Catalog Number MAK033D	1 vl
ADP Standard, 1 µmole Catalog Number MAK033E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

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.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

ADP Assay Buffer – Allow buffer to come to room temperature before use.

ADP Probe – Warm to room temperature to melt frozen solution prior to use. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the ADP Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the ADP Probe Solution 5 to 10-fold with ADP Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

ADP Converter and ADP Developer Mix – Reconstitute each in 220 µL of ADP Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light at –20 °C. Use within 2 months of reconstitution.

ADP Standard – Reconstitute in 100 µL of water to generate a 10 mM (10 nmole/µL) stock solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

ADP Standards for Colorimetric Detection
Dilute 10 µL of the 10 mM (10 nmole/µL) ADP Standard Solution with 90 µL of ADP Assay Buffer to prepare a Will dow1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM ADP standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add ADP Assay Buffer to each well to bring the volume to 50 µL.

ADP Standards for Fluorometric Detection
Dilute 10 µL of the 10 mM (10 nmole/µL) ADP Standard Solution with 990 µL of ADP Assay Buffer to prepare a 0.1 mM (0.1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM ADP standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add ADP Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples can be measured directly.

Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µL of the ADP Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Bring samples to a final volume of 50 µL with ADP Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Pyruvate present in samples will generate increased background. If pyruvate may be present in samples, it is recommended to include a blank sample for each sample by omitting the ADP Converter in the Reaction Mix.

Assay Reaction
1. Set up the Master Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Master Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Blank Sample	Samples and Standards
ADP Assay Buffer	46 µL	44 µL
ADP Probe	2 µL	2 µL
ADP Converter	–	2 µL
ADP Developer	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Cover the plate tightly and protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) ADP Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate ADP standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of ADP present in the sample may be determined from the standard curve.

Concentration of ADP

$$S_a/S_v = C$$

S_a = Amount of ADP in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of ADP in sample

ADP molecular weight: 427.2 g/mole

Sample Calculation

Amount of ADP (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of ADP in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 427.2 \text{ ng/nmole} = 49.9 \text{ ng}/\mu\text{L}$$

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. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the ADP Assay Buffer
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	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	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	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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