Amplite® Universal Fluorimetric Kinase Assay Kit *Red Fluorescence*

Catalog number: 31001, 31002
Unit size: 250 Tests, 500 Tests

Component	Storage	Amount (Cat No. 31001)	Amount (Cat No. 31002)
Component A: ADP Sensor Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (5 mL)	Discontinued
Component B1: ADP Sensor I (Light-sensitive)	Freeze (< -15 °C), Minimize light exposure	1 vial powder	Discontinued
Component B2: ADP Sensor II	Freeze (< -15 °C), Minimize light exposure	1 vial (2.5 mL)	Discontinued
Component B3: DMSO	Freeze (< -15 °C)	1 vial (100 µL)	Discontinued
Component C: ADP Standard	Freeze (< -15 °C), Minimize light exposure	1 vial	Discontinued
Component D: ADP Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (10 mL)	Discontinued

OVERVIEW

Most of commercial protein kinase assay kits are either based on monitoring of phosphopeptide formation or ATP deletion. For the kinase assay kits that are based on detection of phosphopeptides one has to spend time and efforts to identify an optimized peptide substrate while the ATP depletion method suffers various interferences due to the use of luciferase that are inhibited or activated by various biological compounds. The Amplite® Universal Kinase Assay Kit is based on the monitoring of ADP formation, which is directly proportional to enzyme phoshphotransferase activity and is measured fluorimetricaly. This kit provides a fast, simple, and homogeneous assay for measure kinases activities. The characteristics of its high sensitivity (<0.2 uM ADP), broad ATP tolerance (1-300 uM), non-antibody based, non-radioactive and no-wash method to detect the amount of ADP produced as a result of enzyme activity make it an ideal kit for determining kinase Michaelis-Menten kinetics and for screening and identifying kinase inhibitors. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required.

AT A GLANCE

Protocol Summary

1. Run kinase reaction (20 µL)
2. Add ADP Sensor Buffer (20 µL)
3. Add ADP Sensor working solution (10 µL)
4. Incubate at room temperature for 15 minutes - 1 hour
5. Monitor fluorescence Intensity at Ex/Em = 540/590 nm (Cutoff = 570nm)

Important Note

Thaw all the six components at room temperature before use. Black plates are strongly recommended to achieve the best results. The ADP assay should be run at pH from 6.5 to 7.4. Avoid direct exposure of ADP Sensor I (Component B1) to light. Avoid potential ADP contamination from exogenous biological sources.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

ADP Sensor I stock solution (50X)

Add 50 µL DMSO (Component B3) into vial of ADP Sensor 1 (Component B1) to make 50X ADP Sensor I stock solution.

ADP standard solution (300 mM)

Add 100 µL of ddH_2O into ADP Standard (Component C) to make a 300 mM ADP standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/31001

ADP standard

Take 300 mM ADP standard solution and dilute 10,000X in kinase reaction buffer to make 30 µM ADP standard solution. Take 30 µM ADP standard solution and perform 1:3 serial dilution in kinase reaction buffer to get serially diluted of ADP standard solution. Note: Make serial dilutions of ADP standard in the kinase reaction buffer by including a sample without ADP for measuring background fluoresence.

PREPARATION OF WORKING SOLUTION

Add 50 µL of 50X ADP Sensor I stock solution into vial of ADP Sensor II (Component B2) to make ADP Sensor working solution.

Note: The ADP Sensor working solution is not stable, make fresh as needed.

SAMPLE EXPERIMENTAL PROTOCOL

Run Kinase reaction (Reagents are not provided for this step):

1. Prepare 20 µL (or 10 µL for 384-well plate) of kinase reaction solution/well as desired. The components of kinase reaction should be optimized as needed (e.g., an optimized buffer system might be required for a specific kinase reaction). In most cases, ADP assay buffer (Component D) can also be used to run kinase reaction if you do not have the optimized kinase buffer.
2. The Amplite™ Fluorimetric Kinase Assay Kit is used to determine the ADP formation.

Run Amplite™ ADP assay:

1. Add 20 µL of ADP Sensor Buffer (Component A) and 10 µL of ADP Sensor working solution into each well filled with the 20 µL kinase reaction solution to make the total ADP assay volume of 50 µL/well. For a 384-well plate, add 10 µL of ADP Sensor Buffer (Component A) and 5 µL of ADP Sensor into each well filled with the 10 µL kinase reaction solution to make the total ADP assay volume of 25 µL/well.
2. Incubate the reaction mixture at room temperature for 15 minutes to 1 hour.

3. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

Generate an ADP calibration curve (Not required for the screening of kinase inhibitors):

1. Add 20 µL (for a 96-well plate) or 10 µL (for a 384-well plate)/well of ADP Sensor Buffer (Component A) and 10 µL (for a 96-well plate) or 5 µL (for a 384-well plate) of ADP Sensor into each well of serially diluted ADP standards to make the total volume of 50 µL (for a 96-well plate) or 25 µL (for a 384-well plate) for each reaction.
2. Incubate the reaction mixture at room temperature for 15 minutes to 1 hour.
3. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).
4. Generate an ADP standard curve.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ADP Dose samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

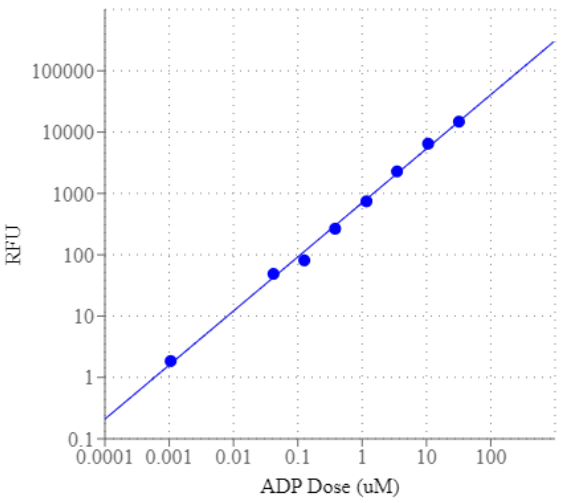

Figure 1. ADP dose response was measured with Amplite® Universal Fluorimetric Kinase Assay Kit in a solid black 384-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

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