

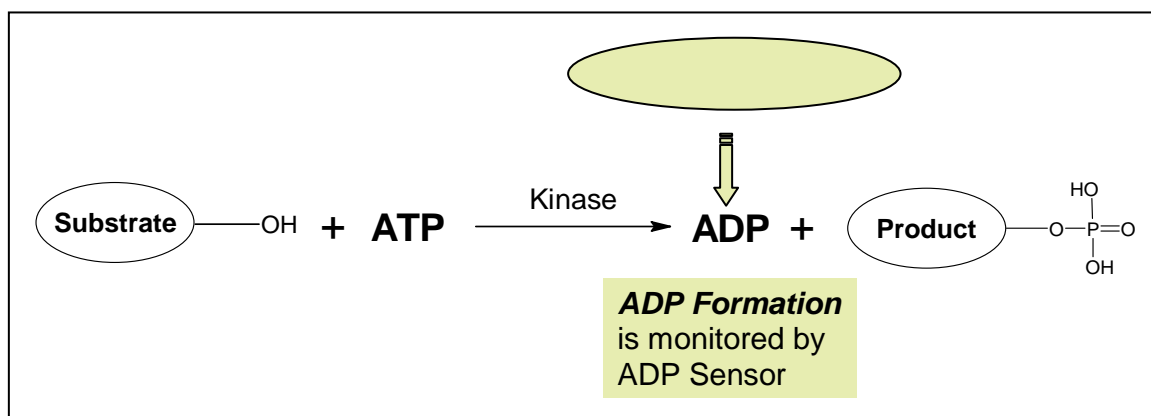
Amplite™ Universal Fluorimetric Kinase Assay Kit

Red Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 31001 (250 assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

Protein kinases are the enzymes that transfer a phosphate group from a phosphate donor to an acceptor amino acid in a substrate protein. Kinases are of great interest to researchers involved in drug discovery. Most of the commercial protein kinase assay kits are based on monitoring either the phosphopeptide formation or the ATP depletion. For the kinase assay kits that are based on the 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 Fluorimetric Kinase Assay Kit is based on monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This enzyme-coupled kit provides a fast, simple, and homogeneous assay to measure kinase activities. It is a non-radioactive and no wash method to detect the amount of ADP produced from enzyme reaction. Its characteristics of high sensitivity (<0.3 μM ADP) and broad ATP tolerance (1-300 μM) 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 without a separation step.

Kit Key Features

Universal:	Can be used for any kinases that used ATP as phosphate donor.
Continuous:	Easily adapted to automation without mixing or separation.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.
Use of Native Substrates:	Substrates can be proteins, peptides or sugars.
Large Range of ATP Tolerance:	ATP can be used from 1-300 μM.
Non-Antibody-Based:	No antibody is used in the kit

Kit Components

Components	Amount
Component A: ADP Sensor Buffer	1 vial (5 mL)
Component B1: ADP Sensor I (Light-sensitive)	1 vial powder
Component B2: ADP Sensor II	1 vial (2.5 mL)
Component B3: DMSO	1 vial (100 µL)
Component C: ADP Standard	1 vial
Component D: ADP Assay Buffer	1 vial (10 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

**Run kinase reaction (20 µL) → Add ADP Sensor Buffer (20 µL) → Add ADP Sensor (10 µL)
→ Incubate at room temperature for 15 minutes - 1 hour → Monitor fluorescence
Intensity at Ex/Em = 540/590 nm (cut off 570nm)**

1. Prepare samples:

- 1.1 Thaw all the six components at room temperature before use.
- 1.2 Avoid direct exposure of ADP Sensor I (Component B1) to light.
Note: Aliquot and store the unused ADP Sensor Buffer (Component A) and 50x ADP Sensor I stock solution (from 3.1) at -20 °C. Avoid repeated freeze/thaw cycles and potential ADP contamination from exogenous biological sources.
- 1.3 Black plates are strongly recommended to achieve the best results.

2. Run kinase reaction (Reagents are not provided for this step):

Warning: The ADP Sensor is unstable in the presence of thiols such as DTT and β -mercaptoethanol. Final thiol concentration higher than 10 µM would significantly decrease the assay dynamic range.

- 2.1 Prepare 20µL (or 10µL 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).
- 2.2 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.
- 2.3 The Amplitude™ Fluorimetric Kinase Assay Kit is used to determine the ADP formation.

3. Run Amplitude™ ADP assay:

Warning: The ADP assay should be run at pH from 6.5 to 7.4.

- 3.1 Make 50 X ADP Sensor I stock solution by adding 50 µL DMSO (Component B3) into vial of ADP Sensor I (Component B1).
Note: Aliquot unused 50 X ADP Sensor I DMSO stock solution, store at -20°C, protect from light
- 3.2 Make ADP Sensor by adding 50 µL of 50 X ADP Sensor I stock solution (from Step 3.1) into vial of ADP Sensor II (Component B2).
Note: The reconstituted ADP sensor is not stable, make fresh as needed.
- 3.3 Add 20 µL (or 10µL for 384-well plate) of ADP Sensor Buffer (Component A) and 10 µL (or 10µL for 384-well plate) of ADP Sensor (from Step 3.2) into each well filled with the 20 µL (or 10µL for 384-well plate) kinase reaction solution (see Step 2.1) to make the total ADP assay volume of 50 µL/well (or 25µL for 384-well plate).

- 3.4 Incubate the reaction mixture at room temperature for 15 minutes to 1 hour.
- 3.5 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm.

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

Note: An ADP standard curve can be generated as described below.

- 4.1 Add 100 μL of ddH₂O into ADP Standard (Component C) to make a 300 mM ADP stock solution. Make serial dilutions of ADP standard in the kinase reaction buffer by including a sample without ADP for measuring background fluorescence.
- Note: Typically, ADP concentrations ranging from 0.05 to 30 μM are appropriate.*
- 4.2 Add the same amount of the serially diluted ADP standards into an empty plate (20 μL /well for a 96-well plate, 10 μL /well for a 384-well plate).
- 4.3 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 (from Step 3.2) into each well of serially diluted ADP standards (from Step 4.2) to make the total volume of 50 μL (for a 96-well plate) or 25 μL (for a 384-well plate) for each reaction.
- 4.4 Incubate the reaction mixture at room temperature for 15 minutes to 1 hour.
- 4.5 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540 /590 nm (Cut off 570 nm).
- 4.6 Generate an ADP standard curve.

Data Analysis

The fluorescence in blank wells (with the kinase buffer only) is used as a control, and is subtracted from the values for those wells with the kinase reactions. An ADP calibration curve is shown in Figure 1 and a protein kinase A reaction curve is shown in Figure 2. *Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

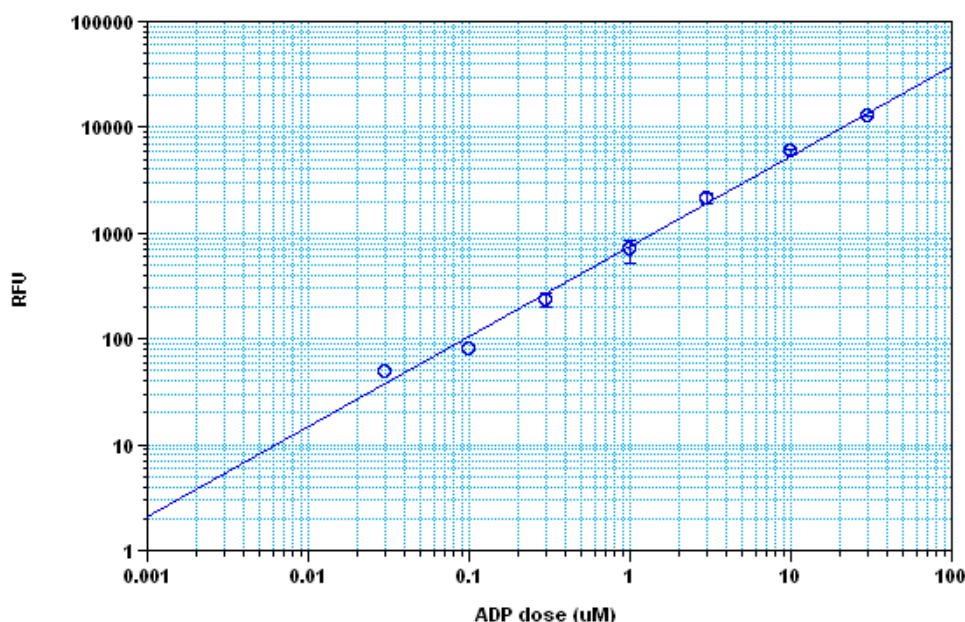


Figure1. ADP dose response was measured with the Amplitude™ Fluorimetric Kinase Assay Kit in a solid black 384-well plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 μM ADP can be detected with 15, 30 minutes and 1 hour incubation (Z' factor =0.65).

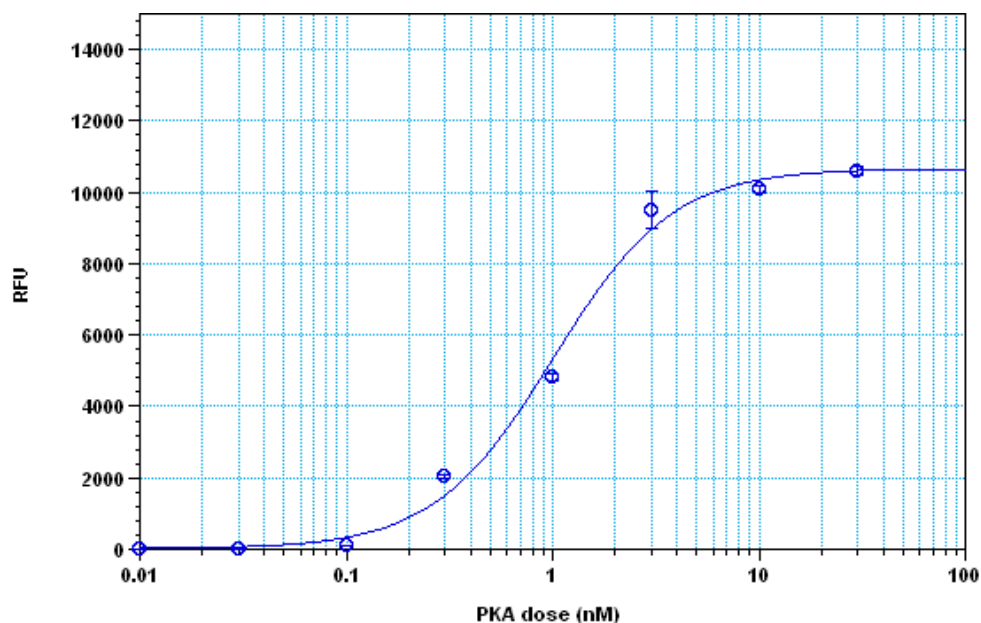


Figure 2. The detection of protein kinase A with the Amplite™ Fluorimetric Kinase Assay Kit. The kinase was incubated in the presence of ATP and kemptide peptide substrate for 30 minutes, and ADP generation was detected after 30 minutes incubation using the Amplite™ Fluorimetric Kinase Assay Kit.

References

1. Cohen P (2000). The regulation of protein function by multisite phosphorylation—a 25 year update. *Trends Biochem Sci* **25**, 596-601.
2. Whitmarsh AJ and Davis RJ (2000). Regulation of transcription factor function by phosphorylation. *Cell Mol Life Sci* **57**, 1172-83.
3. Hunter T (1998). The role of tyrosine phosphorylation in cell growth and disease. *Harvey Lect* **94**, 81-119.
4. McCubrey JA, *et al.* (2000). Serine/threonine phosphorylation in cytokine signal transduction. *Leukemia* **14**, 9-21.
5. Salli U, *et al.* (2000). Phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) protein is associated with bovine luteal oxytocin exocytosis. *Biol Reprod* **63**, 12-20.
6. Parekh DB, *et al.* (2000). Multiple pathways control protein kinase C phosphorylation. *Embo J* **19**, 496-503.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.