

# PhosphoWorks™ Fluorimetric ADP Assay Kit

## \*Red Fluorescence\*

Catalog number: 21655  
Unit size: 100 Tests

Component	Storage	Amount
Component A: ADP Sensor Buffer	Freeze (<-15 °C), Minimize light exposure	1 vial (2 mL)
Component B1: ADP Sensor I (Light-sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial powder
Component B2: ADP Sensor II	Freeze (<-15 °C), Minimize light exposure	1 vial (1 mL)
Component B3: DMSO	Freeze (<-15 °C)	1 vial (100 µL)
Component C: ADP Standard	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: ADP Assay Buffer	Freeze (<-15 °C), Minimize light exposure	1 vial (10 mL)

### OVERVIEW

ADP is involved in many biological reactions such as protein kinases. Our ADP assay kit can be used for assaying protein kinase reactions universally by monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This kit provides a fast, simple and homogeneous assay for measuring ADP formation or depletion. The assay is continuous, and can be easily adapted to automation. The kit is convenient, requiring minimal hands-on time. Protein kinases are of interest to researchers involved in drug discovery due to their broad relevance to diseases such as cancer and other proliferative diseases, inflammatory diseases, metabolic disorders and neurological diseases. Most of commercial protein kinase assay kits are either based on monitoring of phosphopeptide formation or ATP depletion. Our ADP assay kit is more robust for assaying protein kinases than most of commercial kinase assay kits.

### AT A GLANCE

#### Protocol summary

1. Run kinase reaction (20 µL)
2. Add ADP Sensor Buffer (20 µL)
3. Add ADP Sensor (10 µL)
4. Incubate at room temperature for 15 minutes - 1 hour
5. Monitor fluorescence intensity

**Important** Thaw all the six components at room temperature before use. Avoid direct exposure of ADP Sensor I (Component B1) to light.

### KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 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.*

1. **ADP Sensor I stock solution (50X):**  
Add 20 µL DMSO (Component B3) into vial of ADP Sensor I (Component B1).
2. **ADP Sensor stock solution (1X):**  
Add 20 µL of 50X ADP Sensor I stock solution into vial of ADP Sensor II (Component B2).
3. **ADP standard solution (300 mM):**  
Add 100 µL of H<sub>2</sub>O into ADP Standard (Component C) to make a 300 mM ADP stock solution.

### PREPARATION OF STANDARD SOLUTION

#### ADP standard

For convenience, use the Serial Dilution Planner:  
<https://www.aatbio.com/tools/serial-dilution/21655>

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.

### SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of ADP standards and test samples in a solid black 96-well microplate. SD = ADP standard (SD1 - SD7, 0.05 to 30 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

**Table 2.** Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	20 µL	serial dilution (0.05 to 30 µM)
BL	20 µL	ADP assay buffer
TS	20 µL	kinase reaction

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

1. Prepare 20 µL of kinase reaction solution 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 Amplitude™ Fluorimetric ADP Assay Kit is used to determine the ADP

formation. The ADP Sensor is unstable in the presence of thiols such as DTT and  $\beta$ -mercaptoethanol. Final thiol concentration higher than 10  $\mu$ M would significantly decrease the assay dynamic range.

**Run Amplitude™ ADP assay:**

1. Add 20  $\mu$ L of ADP Sensor Buffer (Component A) and 10  $\mu$ L of ADP Sensor stock solution into each well filled with the 20  $\mu$ L kinase reaction solution to make the total ADP assay volume of 50  $\mu$ L/well.

**Note** The ADP Sensor is unstable in the presence of thiols such as DTT and  $\beta$ -mercaptoethanol. Final thiol concentration higher than 10  $\mu$ M would significantly decrease the assay dynamic range.

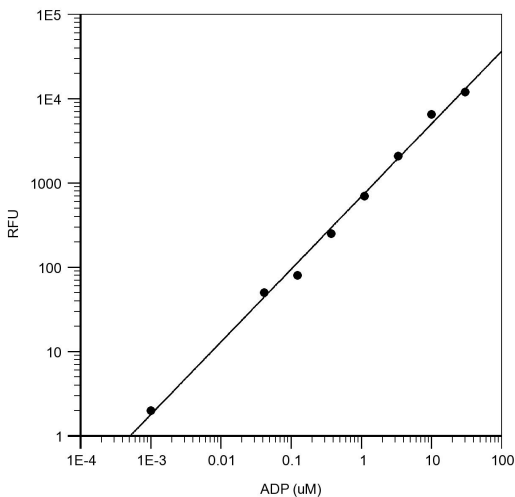
**Note** The ADP assay should be run at pH from 6.5 to 7.4.

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.

**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 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 PhosphoWorks™ Fluorimetric ADP Assay Kit in a solid black 384-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

**DISCLAIMER**

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