ADP Hunter™ Plus Assay
Product Code: 90-0083 Series

FLUORESCENCE INTENSITY DETECTION

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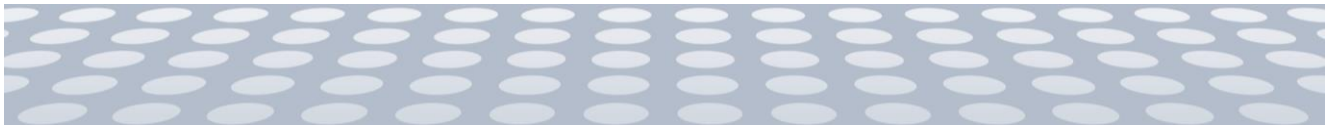
Read the entire product insert fully before beginning the assay.



For additional information or Technical Support, contact DiscoveRx or visit the website.

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INTENDED USE

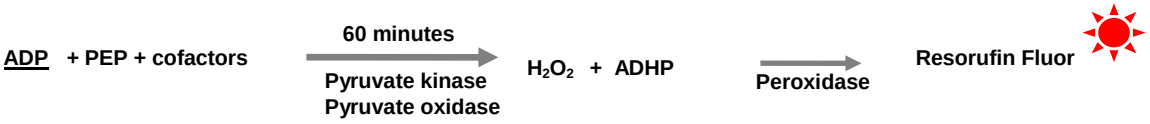
ADP Hunter™ Plus is a biochemical assay to measure the accumulation of ADP, a product of kinase enzyme activity. ADP Hunter Plus is specifically designed for high throughput screening of kinase inhibitors, and is compatible with both peptide substrates and whole proteins. The assay has been designed for research use in 384-well microplates, but can also be run in additional microplate formats.

TECHNOLOGY PRINCIPLE

ADP Hunter Plus is a homogeneous assay for the measurement of ADP, a universal product of kinase activity. Unlike alternative generic approaches that monitor the depletion of ATP from a kinase reaction, this method follows the product of the reaction, and offers a convenient gain-of-signal assay format. This kinase assay is a generic, non-radioactive method that does not require use of antibody. In the ADP Hunter Plus assay a coupled enzyme reaction system is used to generate hydrogen peroxide from ADP. Hydrogen peroxide when combined with ADHP (fluorescent dye precursor) in the presence of peroxidase generates the fluorescently active resorufin dye. To allow for automation, a Stop Solution is also provided for added signal and background stabilization.

Step 1: ***Kinase Reaction***

Step 2: ***ADP Detection***



STORAGE CONDITIONS

Upon arrival, store reagents at -20°C. Thaw reagents at room temperature before use, and after thawing, store reagents for up to 1 week at 2-8°C. For longer term storage, aliquots of all the components must be re-frozen at -20°C. Equilibrate reagents to ambient room temperature before use.

MATERIALS PROVIDED

ADP Hunter Plus kit components are as follows:

Product Code		90-0083	90-0083 M	90-0083 L	90-0083 XL
Test points in full volume 384-well format		**800**	**2,400**	**10,000**	**50,000**
Kit Components					
1	ADP Hunter Plus Reagent A*	8 mL	3 x 8 mL	100 mL	500 mL
2	ADP Hunter Plus Reagent B*	16 mL	3 x 16 mL	200 mL	1000 mL
3	ADP Hunter Plus Stop Solution	4 mL	3 x 4 mL	50 mL	250 mL
4	ADP Hunter Plus Standard (225 µM)	2 mL	3 x 2 mL	25 mL	25 mL
5	ADP Hunter Plus Assay Buffer	20 mL	3 x 20 mL	250 mL	2 x 625 mL

Note: The Assay Buffer is provided for convenience. This buffer may be used as the kinase reaction buffer, to prepare the substrate and/or the kinase, or as a base to add any other cofactors and compounds required for kinase reaction. The assay buffer is pH 7.4 containing 15 mM HEPES, 20 mM NaCl, 1 mM EGTA, 0.02% Tween 20, 10 mM $MgCl_2$, and 0.1 mg/mL BGG (bovine-γ-globulins).

* Reagents A and B upon thawing may show some particulate matter. This does not affect the performance of the assay. If required, reagents can be centrifuged or filtered through 0.2 micron filters to remove the particulate matter.

MATERIALS NOT PROVIDED

The following additional materials are required:

Equipment	Materials
• 384-well black microplates • Pipets and pipet tips • Fluorescence intensity reader • Filter wavelengths required for Resorufin Peak Excitation wavelength: 530 nm Peak Emission wavelength: 590 nm	• Kinase enzyme • Kinase substrate • ATP (recommended DiscoveRx, Catalog # 90-0099) • Other cofactors and additives required for kinase reaction components such as phospholipids, calcium, etc.

GUIDELINES FOR USE

The assay consists of three steps, step 1 is the kinase reaction, step 2 is the addition of the detection reagents, and step 3 is the addition of the assay stop solution.

Kinase Reaction: The kinase reaction includes a purified or semi-purified active kinase, a substrate (peptide or protein), ATP, other appropriate additives and cofactors, compounds, all in an appropriate kinase reaction buffer.

Kinase Reaction Buffer: An assay buffer is provided which may be used as the kinase reaction buffer. If using a different kinase reaction buffer, it is imperative to dilute the ADP standard in your buffer and test for any interference with the detection reagents. The assay is optimal in 6.0-8.0 pH range and can tolerate the following concentrations of commonly used components of the kinase reaction. The concentrations here refer to final kinase reaction concentrations:

ATP	≤ 200 µM	EDTA	≤ 10 mM	NaCl	≤ 150 mM
BGG	≤ 2 mg/mL	EGTA	≤ 10 mM	$MgCl_2$	≤ 10 mM
DMSO	≤ 5 %	Glycerol	≤ 5%	Sodium Orthovanadate	≤ 2 mM
DTT	≤ 2 mM	HEPES	≤ 15 mM	β–mercaptoethanol	≤ 0.01%

Kinase Reaction optimization: Components of the kinase reaction need to be optimized for each kinase and may need to be verified with new batches of kinase due to variability in enzyme activity. For optimal performance always titrate the following components prior to running a full experiment:

- Substrate
- Kinase
- ATP
- Any cofactors needed for kinase activity

Controls: A control without kinase should be included in each experiment and signal from this control may be used for correction of background signal, this control should contain ATP, substrate, and compound or vehicle.

Tips for optimal performance:

- Use the highest quality ATP in performing kinase reactions. Some sources of ATP contain high amounts of contaminating ADP that can significantly increase assay background. We recommend using ATP Gold (DiscoveRx, Cat. # 90-0099). ATP powder should be stored desiccated at -20 °C. Ideally fresh ATP solutions should be used and made up in 100 mM Hepes, pH 7.4. Alternatively stock solution can be aliquoted and stored frozen at -80 °C. Do not freeze-thaw ATP stock solution.
- Equilibrate all detection reagents to room temperature for at least 1 hour before use.
- ADP Reagent A and Reagent B must be added separately. **Do not pre-mix**.
- ***Reagent A must be added before Reagent B for optimal assay performance.***
- Stop Solution must be added last. Do not combine with either Reagent A or Reagent B. The Stop Solution stabilizes signal and background.

REAGENT PREPARATION

For ADP Hunter Plus Standard Curve: Prepare (7) 3-fold serial dilutions of the ADP standard in the assay buffer provided, in polypropylene tubes. The ADP standard concentrations range from 75 to 0.1 µM in the kinase reaction. Use the assay buffer as the zero ADP control.

For the Kinase Reaction: Add cofactors and/or additives to the assay buffer provided or prepare a kinase reaction buffer. The kinase reaction has a final reaction volume of 20 µL (traditional 384-well plate). The kinase reaction should include a purified or semi purified active kinase, a substrate, ATP, and other appropriate additives/cofactors.

ADP Hunter Plus Reagent A: Ready to use, no preparation needed.

ADP Hunter Plus Reagent B: Ready to use, no preparation needed.

ADP Hunter Plus Stop Solution: Ready to use, no preparation needed.

Assay Procedure (Standard Curve)

The table below outlines the volumes and procedure for the ADP standard curve for a full volume 384-well plate format. **For low volume 384-well format, divide all volumes by a factor of 2.**

Full Volume 384-well Plate	ADP Standard
Step 1: Standard Dilutions	20 µL ADP Standard dilutions
Step 2: ADP Detection	a) Add 10 µL Reagent A b) Add 20 µL Reagent B c) Incubate 15-60 minutes at room temperature
Step 3: Stop Solution (optional)	Add 5 µL Stop Solution
Read Fluorescence Intensity signal: Resorufin Excitation wavelength – 530 nm Resorufin Emission wavelength – 590 nm	
Note: The signal may be measured up to 1 hour after addition of Stop Solution. Addition of Stop Solution will result in a 10% reduction in assay window.	

Kinase Assay Procedure

The table below outlines assay volumes and procedure for kinase reactions. The volumes in the table are for a full volume 384-well plate format. **For low volume 384-well format, divide all volumes by a factor of 2.**

Full Volume 384-well Plate	Kinase Reaction
Step 1: Kinase Reaction	20 µL kinase reaction (kinase, substrate, ATP, compound) Kinase Reaction Incubation*
Step 2: ADP Detection	a) Add 10 µL Reagent A b) Add 20 µL Reagent B c) Incubate 15-60 minutes at room temperature
Step 3: Stop Solution (optional)	5 µL Stop Solution
Read Fluorescence Intensity signal: Resorufin Excitation wavelength – 530 nm Resorufin Emission wavelength – 590 nm	
Note: The signal may be measured up to 1 hour after addition of Stop Solution. Addition of Stop Solution will result in a 10% reduction in assay window.	

* The incubation time and temperature for the kinase reaction should be optimized for each kinase. For most kinases, the addition of ADP Hunter Plus reagents will inhibit kinase activity. For kinases not inhibited by the detection reagents, EDTA may be used up to 10 mM.

REPRESENTATIVE DATA

Figure 1:
The ADP Hunter Plus standard curve is illustrated below. (A. Uncorrected data. B. Data after background correction [0 µM ADP]). The dynamic range of the assay is 0.6 µM to 75 µM ADP. The data is shown using a linear fit (r^2 = 0.998).

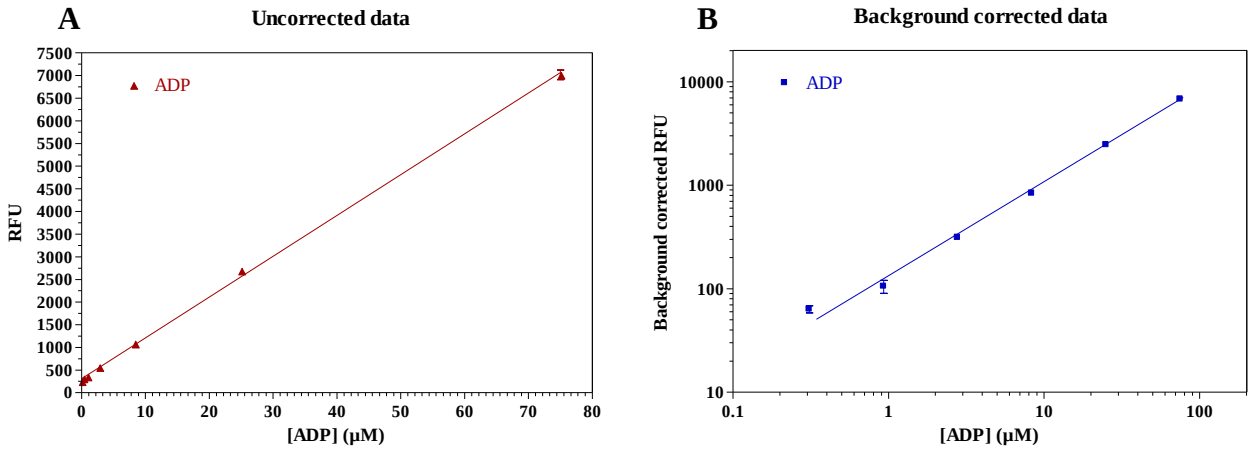


Figure 2:
Kinase activity curves for PKA, CK1 and Jnk2 in the presence of 25 µM ATP. Kinase reactions were performed at 30 °C for 60 minutes.

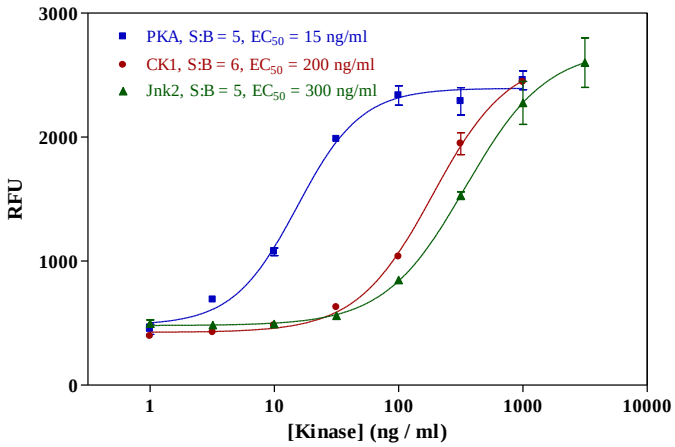
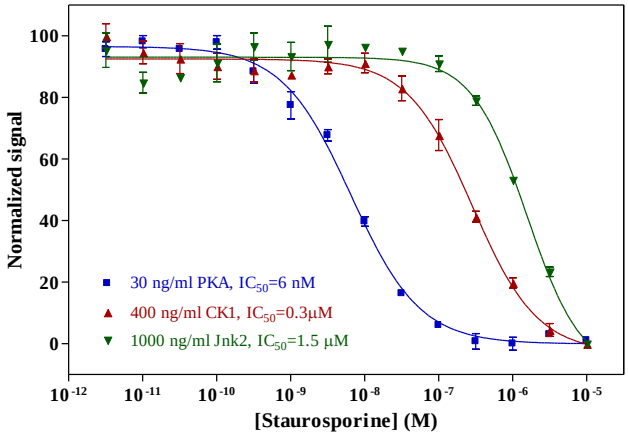


Figure 3:
Staurosporine inhibition of kinase activity in the presence of 25 µM ATP. Kinase reactions were performed at 30 °C for 60 minutes. Data shown is normalized as percentage signal change.

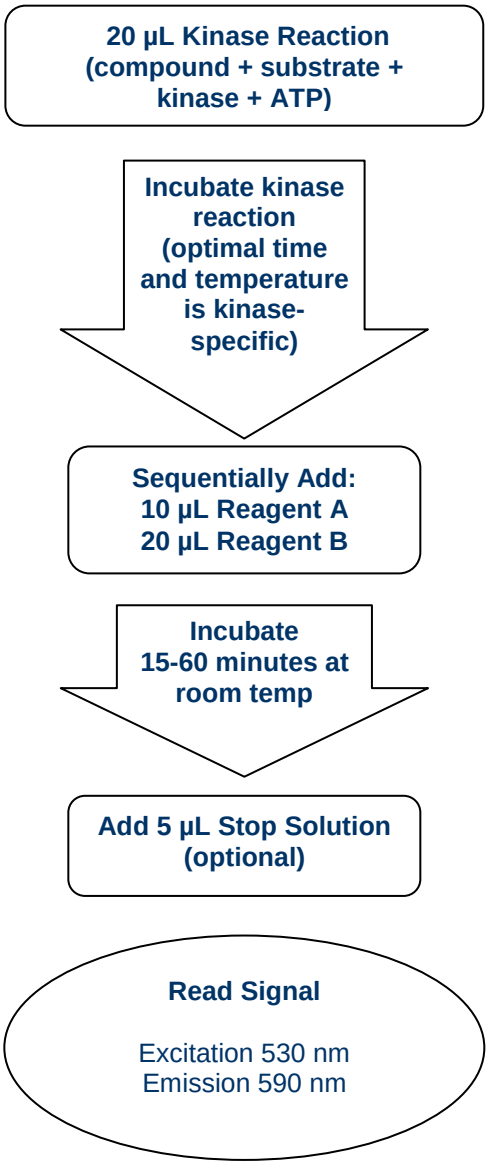


Quick Start Guide

NOTE: Following the quick start guide assumes that you have optimized your kinase reaction conditions, for example, ATP concentration, substrate concentration, and kinase amount*.

The flowchart below outlines assay volumes and procedure for the **full volume 384-well format.** For low volume 384-well format, divide all volumes by a factor of 2. Run appropriate controls with all experiments.

Kinase Assay



*Note: For more detailed procedures and guidelines refer to pages 3 and 4 of this product insert.