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ab138879

Universal Kinase Assay Kit (Fluorometric)

Instructions for Use

For monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity

This product is for research use only and is not intended for diagnostic use.

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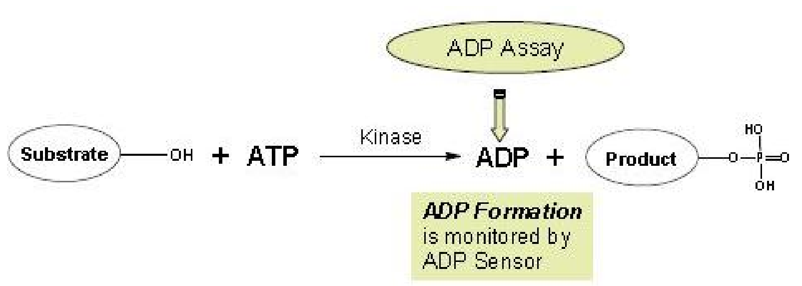
1. Introduction

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.



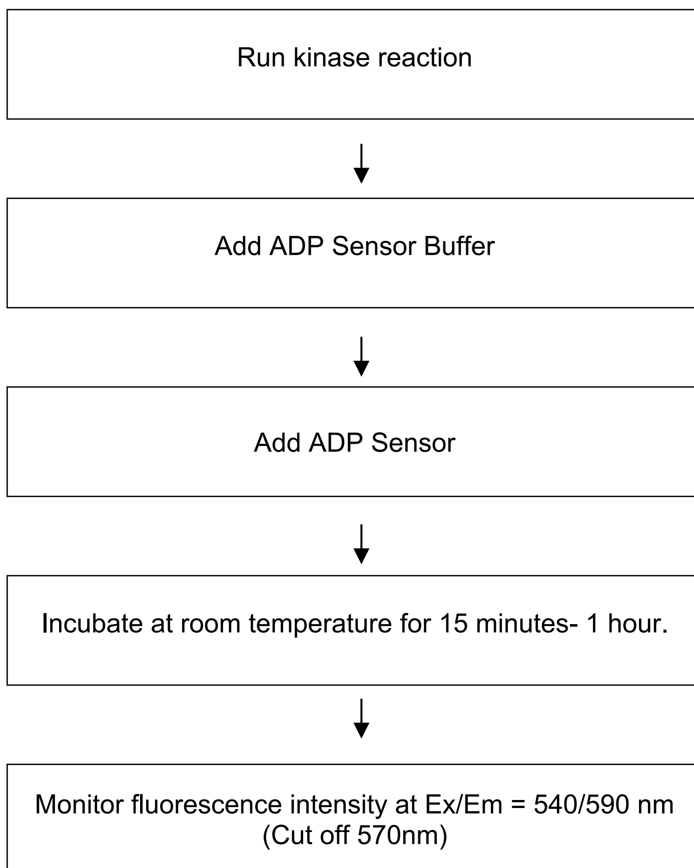
ab138879 is based on monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This enzyme-coupled kit provides a fast, simple, and homogeneous assay to measure kinase activities. It is a non-radioactive and no wash method to detect 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.

2. Protocol Summary

Summary for One 96-well Plate



3. Kit Contents

Components	Amount
ADP Sensor Buffer	5 ml
ADP Sensor I (Light sensitive)	1 vial
ADP Sensor II	2.5 ml
DMSO	100 µl
ADP Standard	1 vial
ADP Assay Buffer	10 ml

4. Storage and Handling

Keep at -20°C (preferably -80°C for long term). Store away from Light.

5. Additional Materials Required

- 96 or 384-well microplates: Solid tissue culture microplates with black walls and clear bottom.
- Fluorescence microplate reader
- Reagents required to run kinase reaction (e.g., an optimized buffer system might be required for a specific kinase reaction).

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of samples

1. Thaw all the components at room temperature before use.

2. Avoid direct exposure of ADP Sensor I to light. *Note: If the entire amount is not used, then aliquot and store unused ADP Sensor Buffer and ADP Sensor I at -20°C. Unused ADP Sensor II should be removed from the vial, aliquoted and stored at -80°C. Avoid repeat freeze/thaw cycles and potential ADP contamination from exogenous biological sources.*

3. Black plates are strongly recommended to achieve the best results.

B. Run kinase reaction (Reagents 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.*

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).

2. In most cases, ADP Assay Buffer can also be used to run kinase reaction if you do not have the optimized kinase buffer.

3. ab138879 can be used to determine the ADP formation.

C. Run ADP Assay:

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

1. Make 50 X ADP Sensor I stock solution by adding 50 ul DMSO into vial of ADP Sensor I.
   Note: Aliquot unused 50 X ADP Sensor 1 DMSO stock solution, store at -20°C, protect from light

2. Make ADP Sensor by adding 50 uL of 50 X ADP Sensor I stock solution (from Step 1) into vial of ADP Sensor II. ADP Sensor is not stable, so it has to be made fresh from ADP Sensor I and II for each assay.

3. Add 20 µl of ADP Sensor Buffer and 10 µl of ADP Sensor into each well filled with 20 µl of kinase reaction solution to make the total ADP assay volume of 50 µl/well.

4. Incubate the reaction mixture at room temperature for 15 minutes to 1 hour.

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

D. Preparation of an ADP calibration curve (Not required for the screening of kinase inhibitors

1. Add 100 µl of H_2O into ADP Standard 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.

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).

3. Add 20 µl of ADP Sensor Buffer and 10 µl of ADP Sensor into each well of serially diluted ADP standards (see D.2 above) to make the total volume of 50 µl for each reaction.

4. Incubate the reaction mixture at room temperature for 15 minutes to 1 hour.

5. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540 /590 nm.

6. Generate an ADP standard curve.

7. 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.

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.

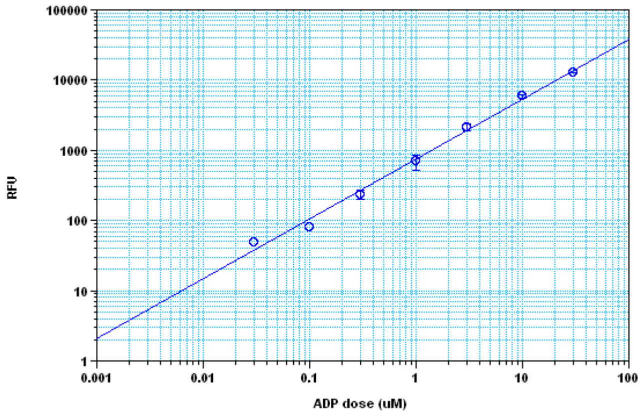


Figure 1. ADP dose response was measured with ab138879 in a solid black 384-well plate using a fluorescence microplate reader. As

low as 0.3 µM ADP can be detected with 15, 30 minutes and 1 hour incubation (Z' factor =0.65).

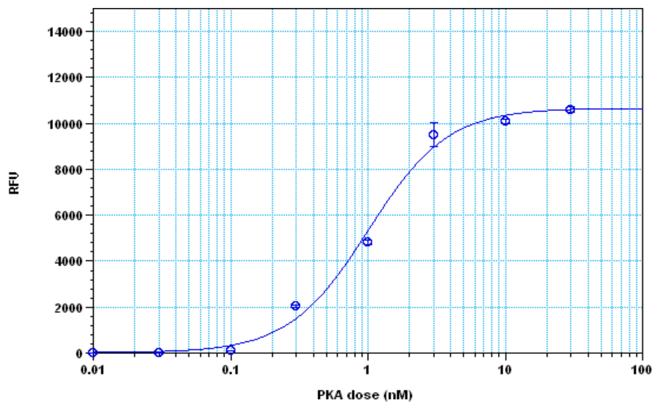


Figure 2. The detection of protein kinase A with ab138879. The kinase was incubated in the presence of ATP and kemptide peptide substrate for 30 minutes, and ADP generation was detected after 30 minutes incubation.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab204708)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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