3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Universal Fluorometric Kinase Assay Kit

Catalog Number **MAK173**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Protein kinases are enzymes that catalyze the transfer of a phosphate group from a phosphate donor to a substrate protein. Kinases are of great interest to researchers due to their role in cell signaling and their roles in disease states.

Most of the commercial protein kinase assay kits are based on monitoring either phosphopeptide formation or ATP depletion. Kinase assays that rely on the detection of phosphopeptides require significant effort to identify an optimized peptide for the specific kinase of interest. The ATP depletion method suffers various interferences due to inhibition or activation of luciferase by compounds found in the biological matrix.

This Universal Fluorimetric Kinase Assay Kit is based on monitoring ADP formation, which is directly proportional to kinase activity. This enzyme-coupled kit provides a fast, efficient, and homogeneous assay to measure kinase activities. This assay kit is a non-radioactive and non-antibody based method to detect the amount of ADP produced from enzyme reaction without a separation step.

This kit demonstrates high sensitivity (<0.3 μM ADP) and broad ATP tolerance (1–300 μM). This kit can be utilized for determining kinase Michaelis-Menten kinetics, and for screening and identifying kinase inhibitors.

Components
The kit is sufficient for 250 assays in 96 well plates.

ADP Sensor Buffer Catalog Number MAK173A	5 mL
ADP Sensor 1 Catalog Number MAK173B1	1 vial
ADP Sensor 2 Catalog Number MAK173B2	2.5 mL
DMSO Catalog Number MAK173B3	100 μL
ADP Standard Catalog Number MAK173C	1 ea
ADP Assay Buffer Catalog Number MAK173D	10 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for best results.
- Fluorescence multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on dry ice and storage at –20 °C, protected from light, is recommended.

Preparation Instructions
Allow reagents to come to room temperature and briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Use ultrapure water for the preparation of reagents.

Avoid direct light exposure to the ADP Sensor Buffer MAK173A. ADP Sensor Buffer MAK173A is unstable in the presence of thiols. A concentration of greater than 10 μM thiol will significantly impact results.

Prepare 300 mM ADP Stock Solution if calibration curve is required:
Note: A calibration curve is not required to monitor relative differences for screening kinase inhibitors.

Add 100 μL of ultrapure water into ADP Standard MAK173C to make a 300 mM ADP Stock Solution.

Prepare ADP Sensor Solution:
Make 50× ADP Sensor I Stock Solution by adding 50 µL of DMSO (MAK173B3) into vial of ADP Sensor 1 (MAK173B1).
Note: Aliquot any unused 50× ADP Sensor I Stock Solution and store at –20 °C protected from light

Make ADP Sensor Solution by adding 50 µL of 50× ADP Sensor I Stock Solution into vial of ADP Sensor II (MAK173B2).

Procedure
All samples and standards should be run in duplicate.

Run Kinase Reaction of Interest
Obtain 20 µL of kinase reaction solution. Perform kinase reaction in an optimized buffer system if one is identified. If an optimal buffer system has not been identified one can use MAK173D ADP Assay Buffer in most cases. Utilize 20 µL of kinase reaction solution per well.

Fluorometric ADP Assay for Kinase Reaction

1. To each well containing 20 µL of kinase reaction solution, add 20 µL of MAK173A ADP Sensor Buffer and 10 µL of ADP Sensor Solution for a total assay volume of 50 µL.

Reagent	Volume
Kinase Reaction Solution	20 µL
ADP Sensor Buffer	20 µL
ADP Sensor Solution	10 µL
Total Assay Volume	50 µL

Note: The ADP Assay should be run at a pH of 6.5–7.4.

2. Incubate the assay mixture at room temperature for 15 minutes to 1 hour.

3. Monitor the fluorescence intensity (λ_{ex} = 540 nm/ λ_{em} = 590nm).

Generate ADP Calibration Curve
Note: A calibration curve is not required to monitor relative differences for screening kinase inhibitors.

1. Make serial dilutions of 300 mM ADP Stock Solution in the kinase reaction buffer.
   Notes: Typically, ADP concentrations ranging from 0.05–30 µM are appropriate.

2. Add 20 µL of each standard to a separate empty well.
   Notes: Utilize one well as a control using 20 µL of water in place of ADP standard solution (zero ADP control).

   The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the zero ADP controls for each data point.

3. To each well containing a ADP Standard or the zero ADP control, add 20 µL of MAK173A ADP Sensor Buffer and 10 µL of ADP Sensor Solution for a total assay volume of 50 µL.

Reagent	Volume
ADP Standard or Control	20 µL
ADP Sensor Buffer	20 µL
ADP Sensor Solution	10 µL
Total Assay Volume	50 µL

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

5. Monitor the fluorescence intensity (λ_{ex} = 540 nm/ λ_{em} = 590nm).

6. Generate a calibration curve

Results
Calculations
The background blank for the assay is the value obtained for the zero ADP control. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the standards to plot a calibration curve.

Note: A new calibration curve must be set up each time the assay is run.

The kinase activity present in the samples may be determined from the calibration curve.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold Reagents	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates
Samples with erratic readings	Samples prepared in an incompatible buffer	Make sure the assay buffer has a pH between 6.5–7.4 and that thiols are present at less than 10 μM.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
	Improperly thawed components	Thaw all components completely and mix gently before use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
Non-linear standard curve	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
Unanticipated results	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

MJM,MAM 05/14-1