Description

The PDK3 Kinase Assay Kit is designed to measure PDK3 activity for screening and profiling applications using ADP-Glo® as a detection reagent. The PDK3 Kinase Assay Kit comes in a convenient 96-well format, with enough purified PDK3, Casein, ATP, and kinase assay buffer for 96 enzyme reactions. The signal is measured using a luminometer or a microplate reader capable of reading chemiluminescence.

Background

The pyruvate dehydrogenase (PDH) complex is a mitochondrial complex that converts pyruvate to acetyl-CoA. It is a primary regulator of glucose metabolism by providing the link between glycolysis and the tricarboxylic acid cycle. The enzymatic activity of PDH is regulated by a phosphorylation/dephosphorylation cycle, in which phosphorylation results in inactivation of PDH. PDK3 inhibits the PDH complex by phosphorylation of the E1 alpha subunit.

PDK3 participates in the metabolic switch of cancer cells; although its significance in tumor malignancy is unknown. PDK3 has been implicated in drug resistance following the treatment of leukemia or colon cancer.

Applications

Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100927	PDK3*	50 µg	-80°C
79334	5x Kinase assay buffer	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
	Casein (10 mg/ml)	250 µl	-20°C
79696	96-well plate, white	1	Room Temp.

*** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.**

Materials Required but Not Supplied

Name	Catalog #
ADP-Glo® Kinase Assay (ADP-Glo reagent and Kinase Detection reagent)	Promega #V6930
Dithiothreitol (DTT 0.5 M)	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw 5x Kinase assay buffer, ATP (500 µM), and Casein (10 mg/ml). Add DTT (0.5 M) to 5x Kinase assay buffer to make a 10 mM concentration (e.g. add 20 µl of 0.5 M DTT to 1 ml 5x Kinase assay buffer).
2. Prepare the Master Mix (12.5 µl per well): N wells x (3 µl of 5x Kinase assay buffer + 0.5 µl of ATP (500 µM) + 2.5 µl of Casein (10 mg/ml) + 6.5 µl of distilled water). Add 12.5 µl of Master Mix to every well.
3. Prepare test inhibitor solution by diluting the stock solution of test compound 1:10 with 1x kinase assay buffer. Note: If inhibitor was dissolved in DMSO, keep DMSO concentration of the Test Inhibitor at ≤10% in the inhibitor solution, as the final DMSO concentration in the reaction should be ≤1%.
4. Add 2.5 µl of Inhibitor solution of each well labeled as "Test Inhibitor."
5. For the "Positive Control" and "Blank," prepare a "Diluent Solution" that contains the same concentration of diluent as the test inhibitor in 1x Kinase assay buffer (for example 10% DMSO in 1x Kinase assay buffer if the inhibitor was dissolved in DMSO), but does not contain inhibitor. Add 2.5 µl of this diluent solution to "Blank" and "Positive Control".
6. Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl water. Three ml of 1x Kinase assay buffer is sufficient for 100 reactions.
7. To the wells designated as "Blank," add 10 µl of 1x Kinase assay buffer.
8. Thaw PDK3 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of PDK3 required for the assay and dilute enzyme to 50 ng/µl with 1x Kinase assay buffer. Store remaining undiluted enzyme in aliquots at -80°C.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5	12.5	12.5
Test inhibitor	-	-	2.5 µl
Diluent solution (no inhibitor)	2.5 µl	2.5 µl	-
1x Kinase assay buffer	10 µl		
PDK3 (50 ng/µl)	-	10 µl	10 µl
Total	25 µl	25 µl	25 µl

Note: PDK3 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

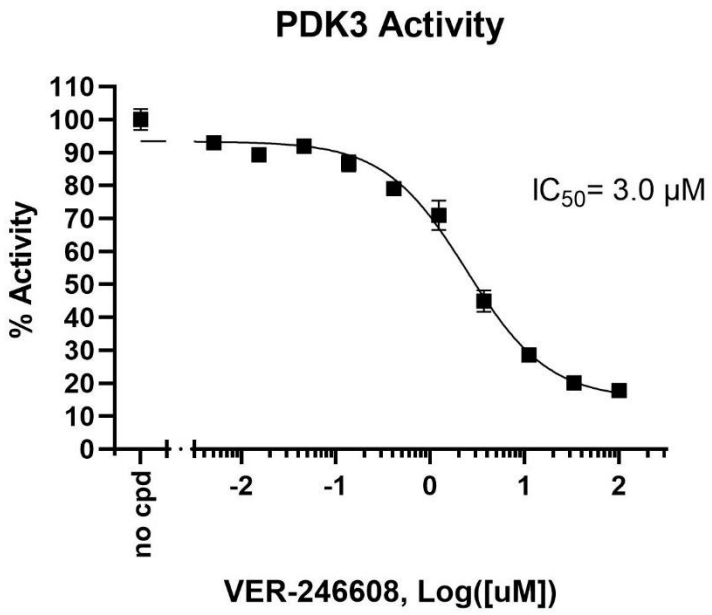
9. Initiate the reaction by adding 10 µl of diluted PDK3 enzyme to the wells designated "Positive Control" and "Test Inhibitor." Incubate at 30°C for 45 minutes.

10. Thaw ADP-Glo reagent (Promega).
11. At the end of the 45 minutes reaction, add 25 µl of ADP-Glo reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 45 minutes.
12. Thaw Kinase Detection reagent (Promega).
13. After the 45-minute reaction, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 45 minutes.
14. Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry. To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results



Inhibition of PDK3 by VER-246608 (Medchem Express #HY-12492), measured using the PDK3 kinase assay kit (Cat. #78286). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General considerations

"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

1. Feng, Lan, *et al.* "miR-497-5p inhibits gastric cancer cell proliferation and growth through targeting PDK3." *Bioscience Reports* 39.9 (2019): BSR20190654.
2. Lu, Chun-Wun, *et al*. "Overexpression of pyruvate dehydrogenase kinase 3 increases drug resistance and early recurrence in colon cancer." *Amer. J. Pathol.* 179.3 (2011): 1405-1414.
3. Mohammad, Taj, *et al.* "Identification of high-affinity inhibitors of pyruvate dehydrogenase kinase-3: Towards therapeutic management of cancer." *J. Biomolec. Struct. Dynam.* 39.2 (2021): 586-594.

Related Products

Products	*Catalog #*	*Size*
PDK3, GST-tag	100927	10 µg
PDK1, GST-tag	40080	10 µg
AKT2, Active, His-tag	40011	10 µg
PDHK1, GST-tag	40501	20 µg
PKLR Var2 (PKL), His-tag	40502	20 µg
PKM2 (PKM2/Variant 1), His-tag	50295	20 µg
Mouse PKM2, His-tag	50296	20 µg