6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

PIM3 Assay Kit

Catalog #79886
96 Reactions

Background: PIM kinases (PIM1, PIM2 and PIM3) are a family of serine/threonine protein kinases that play crucial roles in cell survival, proliferation, and drug resistance. PIM kinases are overexpressed in several tumors and promote growth and survival of malignant cells through cell cycle regulation and/or inhibition of apoptosis. Recently, PIM kinases were identified as a potential therapeutic target for precision medicine of advanced cancer.

DESCRIPTION: The *PIM3 Assay Kit* is designed to measure PIM3 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *PIM3 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant PIM3 enzyme, PIM substrate (S6Ktide), ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
41108	PIM3	3 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
79884	PIM substrate (S6Ktide, 10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 1 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

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

STABILITY: Up to 6 months when stored as recommended.

REFERENCE:
1. Jeyapal G. P., *et al., Anticancer Agents Med Chem*, 2018, **18(8):** 1100-1114
2. Asati V., *et al., Eur J Med Chem*. 2019, **172**: 95-108

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **PIM Substrate (S6Ktide, 10 mg/ml)**.
(Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer**)

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **PIM Substrate (S6Ktide, 10 mg/ml)** + 17 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
PIM Substrate (S6Ktide, 10 mg/ml)	1 µl	1 µl	1 µl
Distilled Water	17 µl	17 µl	17 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (*e.g.* 10% DMSO(aq))	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
PIM3 (1.5 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Prepare 10X concentrated inhibitor in an aqueous-based solution. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution to the assay to make a 1% DMSO concentration in the final reaction mixture.*

4) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer).

5) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions. Dilute only enough **5x kinase assay buffer** as required for the assay.

6) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

7) Thaw **PIM3** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **PIM3** required for the assay and

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dilute enzyme to ~1.25 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C.

Note: PIM3 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

8) Initiate reaction by adding 20 µl of diluted **PIM3** to the wells designated "Positive Control" and "Test Inhibitor." Incubate at 30˚C for 45 minutes.

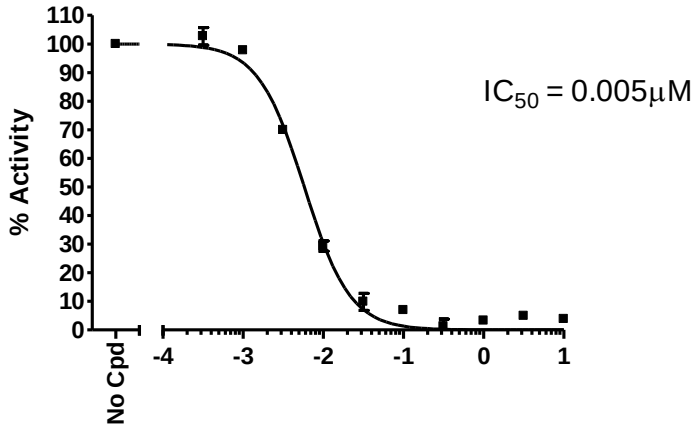
9) Thaw Kinase-Glo Max reagent.

10) After the 45 minutes reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

11) Measure luminescence using a microplate reader capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Example of Assay Results:

PIM3 Activity



Inhibition of PIM3 by AZD1208 measured using the PIM3 assay kit (BPS Bioscience #79886). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
PIM1, Active, GST-tag	41107	10 µg
PIM2, Active, GST-tag	40153	10 µg
PIM3, Active, GST-tag	41108	10 µg
Kinase Buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl

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