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Data Sheet

RIPK1 Kinase Assay Kit (384-well)

Catalog #79689

Background: RIPK1 is a member of the receptor-interacting protein kinase (RIP) family. It plays an important role in cell survival as well as cell death, including apoptosis and necroptosis. RIPK1 is highly expressed in Alzheimer's disease (AD) brains. Importantly, it was shown that inhibition of RIPK1 results in decreased amyloid and inflammation levels as well as accelerated amyloid beta peptide (Aβ) degradation by microglia *in vitro*.

Description: The *RIPK1 Kinase Assay Kit* is designed to measure RIPK1 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *RIPK1 Kinase Assay Kit* comes in a convenient 384-well format, with enough purified recombinant RIPK1 enzyme, RIPK1 substrate, ATP and kinase assay buffer for 400 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40371	RIPK1	2 X 10 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	2 X 1.5 ml	-20°C	
79686	ATP (500 µM)	2 X 100 µl	-20°C	
	RIPK1 substrate (MBP)	2 X 100 µl	-20°C	
	384-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo® Kinase Assay (Promega #V6930)
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.

CONTRAINDICATION: Avoid >1% DMSO

REFERENCE:
Ofengeim D., *et al. Proc. Natl. Acad. Sci. USA* **114(41):** E8788-E8797 (2017)

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

1) Thaw **5x Kinase assay buffer, ATP** and **RIPK1 substrate (MBP).** (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.** Prepare only enough 5x Kinase assay buffer with DTT as required for the assay, as any excess 5x kinase buffer/DTT cannot be stored and should be discarded.)

2) Calculate the amount of ATP (150 µM) required according to the table below, and dilute 500 µM ATP to 150 µM in distilled water (*e.g.* add 150 µl of **500 µM ATP** to 350 µl water to prepare 500 µl of 150 µM ATP).

3) Prepare the master mixture (8.5 µl per well): N wells x (2 µl **5x Kinase assay buffer** + 1 µl diluted **ATP (150 µM)** + 0.5 µl **RIPK1 substrate (MBP)** + 5 µl distilled water). Add 8.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	2 µl	2 µl	2 µl
ATP (150 µM)	1 µl	1 µl	1 µl
RIPK1 substrate (MBP)	0.5 µl	0.5 µl	0.5 µl
Water	5 µl	5 µl	5 µl
Test Inhibitor	–	1.5 µl	–
10% DMSO in water (Inhibitor buffer)	1.5 µl	–	1.5 µl
1x Kinase buffer	–	–	5 µl
RIPK1 (~10 ng/µl)	5 µl	5 µl	–
Total	15 µl	15 µl	15 µl

4) 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 1.5 µl of the 100 µM solution to the assay to make a 1% DMSO concentration in the final reaction mixture.*

5) Add 1.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 1.5 µl of 10% DMSO in water (Inhibitor buffer).

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6) 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.)

7) To the wells designated as "Blank," add 5 µl of **1x Kinase assay buffer**.

8) Thaw **RIPK1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **RIPK1** required for the assay and dilute enzyme to 10 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: RIPK1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

9) Initiate reaction by adding 5 µl of **diluted RIPK1 enzyme** to the wells designated “Positive Control” and "Test Inhibitor Control." Carefully shake the plate well and incubate it at 30˚C for 50 minutes.

10) Thaw ADP-Glo reagent.

11) After the 50 minutes reaction, add 15 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.

13) After the 45 minutes incubation, add 30 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) Measure luminescence using the microplate reader. “Blank” value should be subtracted from all wells.

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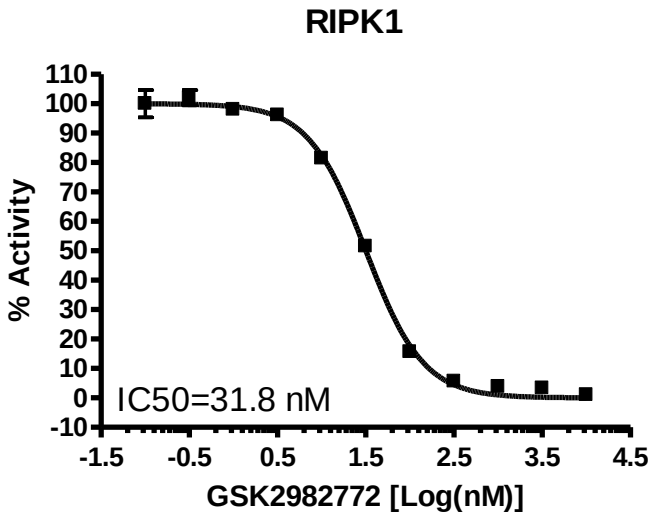
Example of Assay Results:



Inhibition of RIPK1 enzyme by GSK2982772, measured using the *RIPK1 kinase assay kit* (Cat. #79689). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
RIPK1, GST-Th-Tag	40371	10 µg
RIPK2, GST-tag	40173	10 µg
RIPK5, GST-tag	40174	10 µg
LRRK2 (RIPK7), GST-Tag	40842	10 µg
LRRK2 (Y1699C), GST-Tag	40838	10 µg
LRRK2 (Y1699G), GST-Tag	40839	10 µg
LRRK2 (R1441H), GST-Tag	40837	10 µg
LRRK2 (R1441G), GST-Tag	40836	10 µg
LRRK2 (R1441C), GST-Tag	40835	10 µg
LRRK2 (G2385R), GST-Tag	40833	10 µg
LRRK2 (G2019S), GST-Tag	40832	10 µg
LRRK2 (D1994A), GST-Tag	40831	10 µg
LRRK2 (I2020T), GST-Tag	40834	10 µg

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