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Data Sheet

RIPK2 Kinase Assay Kit

Catalog #79737
96 reactions

Background: RIPK2 (receptor-interacting protein kinase 2) has been shown to mediate the activation of pro-inflammatory signals by NODs (Nucleotide-binding oligomerization domain-containing proteins),suggesting RIPK2 may be a promising therapeutic target in autoimmune and inflammatory diseases.

Description: The *RIPK2 Kinase Assay Kit* is designed to measure RIPK2 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *RIPK2 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant RIPK2 enzyme, RIPK substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40173	RIPK2	5 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	
	RIPK substrate (MBP)	100 µl	-20°C	
	96-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: Canning P., *et al. Chem. & Biol.* **22(9):** 1174-1184 (2015)

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

1) Thaw **5x Kinase assay buffer, ATP** and **RIPK 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) Prepare the master mixture (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 1 µl **RIPK substrate (MBP)** + 8 µl distilled water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	3 µl	3 µl	3 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
RIPK substrate (MBP)	1 µl	1 µl	1 µl
Water	8 µl	8 µl	8 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor Buffer (*e.g.* 10% DMSO(aq))	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
RIPK2 (~5 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 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 2.5 µl of the 100 µM solution to the assay to make a 1% DMSO concentration in the final reaction mixture.*

4) Add 2.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 2.5 µl of the same solution without inhibitor (Inhibitor buffer, *e.g.* 10% DMSO(aq)).

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.

6) To the wells designated as "Blank", add 10 µl of **1x Kinase assay buffer**.

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7) Thaw **RIPK2 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **RIPK2** required for the assay and dilute enzyme to 5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: RIPK2 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 10 µl of **diluted RIPK2 enzyme** to the wells designated “Positive Control” and "Test Inhibitor Control". Carefully shake the plate well and incubate it at 30˚C for 45 minutes.

9) Thaw ADP-Glo reagent.

10) After 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.

11) Thaw Kinase Detection reagent.

12) After the 45 minutes incubation, 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.

13) Measure luminescence using the microplate reader. “Blank” value should be subtracted from all wells.

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).

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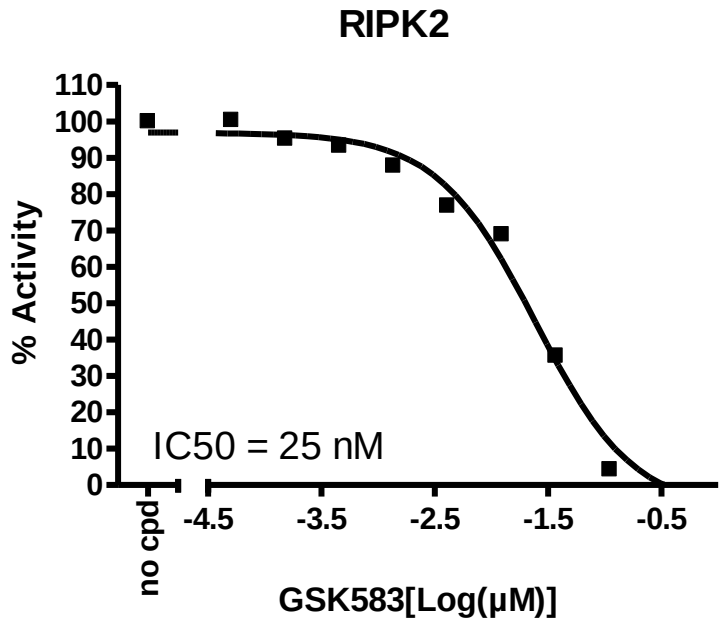
Example of Assay Results:



Inhibition of RIPK2 enzyme by GSK583, measured using the *RIPK2 kinase assay kit* (Cat. #79737). *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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