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Data Sheet

HPK1 Assay Kit

Catalog 79775
96 Reactions

Background: HPK1 (MAP4K1) or Hematopoietic progenitor kinase 1 is a hematopoietic cell-restricted member of the Ste20 serine/threonine kinase super family. It is a tissue-specific upstream activator of the MEKK/JNK/SAPK signaling pathway. HPK1 diminishes T cell receptor (TCR) signaling activity and T cell proliferation by phosphorylating the adaptor protein SLP-76, suggesting HPK1 could be a novel target for anti-tumor immunotherapy.

DESCRIPTION: The *HPK1 Assay Kit* is designed to measure HPK1 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *HPK1 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant HPK1 enzyme, HPK1 substrate (MBP), ATP, and kinase assay buffer for 100 enzyme reactions.
MBP (Myelin Basic Protein) is a non-specific protein substrate that is used as a "universal substrate" for many in-vitro kinase activity assays. This protein is targeted by many serine/threonine kinases at conserved amino acids. We use the dephosphorylated version of the MBP substrate in our assays to determine the kinase-mediated phosphorylation of MBP. Our assays are not suitable for studying autophosphorylation of the kinase due to the presence of the MBP substrate.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40398	HPK1	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	
	MBP (5 mg/ml)	200 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

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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.

REFERENCES:
1. Wu P, *et al., Structure*, 2019, **27**: 125-133.e4
2. Alzabin S, *et al., J. Immunol*. 2009, **182**: 6187-6194
3. Jakob SM, *et al., Blood*, 2013, **121**(20):4184-4194

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

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **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**)

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 2 µl **MBP (5 mg/ml)** + 16 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
MBP (5 mg/ml)	2 µl	2 µl	2 µl
Water	16 µl	16 µl	16 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (*e.g.* 10% DMSO(aq))	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
HPK1 (1.5 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) 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).

4) 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.

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

6) Thaw **HPK1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **HPK1** required for the assay and dilute enzyme to 1.5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C.

   Note: HPK1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

7) Initiate reaction by adding 20 µl of diluted **HPK1** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 50 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 50 minute 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.

10) Measure luminescence using the microplate reader. “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).

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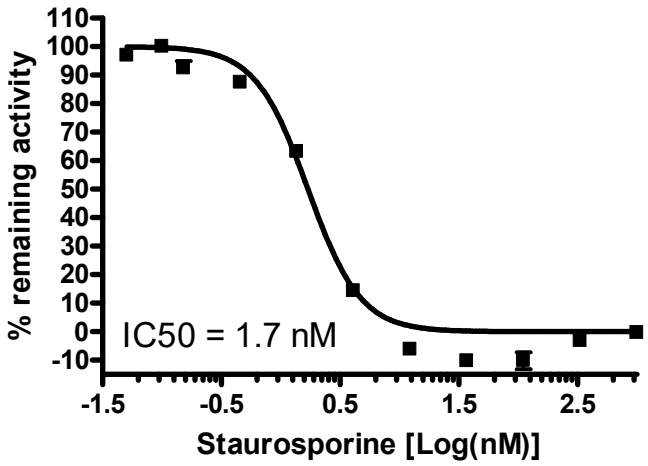
Example of Assay Results:



Inhibition of HPK1 by Staurosporine measured using the HPK1 assay kit (BPS Bioscience #79775). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
HPK1 (MAP4K1), GST-tag, His-Avi-tag	40398	10 µg
GCK (MAP4K2), GST-tag	40107	10 µg
HGK (MAP4K4), GST-tag	40109	10 µg
HGK (MAP4K5), GST-tag	40109	10 µg
MINK1 (MAP4K6), GST-tag	40126	10 µg
TNIK (MAP4K7), GST-tag	79304	10 µg
MBP, His-tag	40535	100 µg