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Description

The Chemi-Verse™ HPK1 Kinase Assay Kit is designed to measure HPK1 (Hematopoietic progenitor kinase 1) serine/threonine kinase activity for screening and profiling applications using ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified HPK1 (amino acids 1-346, kinase domain), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Note: 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.

Background

HPK1 (Hematopoietic progenitor kinase 1), also known as MAP4K1, is a restricted member of the Ste20 serine/threonine kinase super family expressed in the hematopoietic cell lineage, with high expression in T, B and dendritic cells (DCs). It is considered a negative intracellular immune checkpoint as HPK1 diminishes T cell receptor (TCR) signaling activity and T cell proliferation. Cytosolic HPK1 is recruited to the plasma membrane when the TCR is activated, is phosphorylated, then phosphorylates the adaptor protein SLP-76 (SH2 domain containing leukocyte protein of 76 kDa), forming a site for the negative regulator 14-3-3 to bind and destabilize the TCR complex. It also plays a scaffolding role in NF- κ B (nuclear factor kappa light-chain-enhancer of activated B cells) regulation in AICD (activation-induced cell death) and LFAT-1 (lymphocyte function-associated antigen 1) activation. Knock-down or knock-out of HPK1 in animals resulted in improved anti-viral and anti-tumor immunity, suggesting that HPK1 could be a novel target for anti-tumor immunotherapy. The development of small molecule inhibitors specific for HPK1 has not been easy due to its similarity to other MAP4K proteins. Additionally, typical off-target management links to the minimization of effects on crucial functions for life, while in the case of HPK1 one must consider effects on functions that support cancer cell killing. The use of allosteric inhibitors, CRISPR, shRNA, ASO (antisense oligonucleotides) or PROTAC (Proteolytic targeting chimeras) are also interesting alternatives that can be used to target HPK1. Recently, the results of a phase I/II clinical trial for NDI-101150, a selective inhibitor of HPK1, have indicated an increased infiltration of CD8⁺ T cells and DCs into RCC (renal cell carcinoma), showcasing the role and importance of HPK1 in oncology.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40398	HPK1, GST-Tag, His-Avi-Tag (Human)*	3 μ g	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 μ M ATP	50 μ l	-20°C
78514	MBP (5 mg/ml)	100 μ l	-20°C
82545	White 96-well plate	1	Room Temperature

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

Materials Required but Not Supplied

Name	Ordering Information
ADP-Glo™ Kinase Assay DTT (Dithiothreitol), 1M, optional Microplate reader capable of reading luminescence Adjustable micropipettor and sterile tips 30°C incubator	Promega #V6930

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 Principle

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
 - The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
 - We recommend maintaining the diluted protein on ice during use.
 - For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
 - We recommend using GNE-1858 (#82585) or Staurosporine (#27002) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC₅₀ value shown in the validation data below.
 - For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com/serial-dilution-protocol).
1. Thaw **5x Kinase Assay Buffer 1**, **500 μM ATP**, and **MBP (5 mg/ml)**.

*Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 µl of **5x Kinase Assay Buffer 1** with 2,400 µl of distilled water.

*Note: Three (3 ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 1 µl of MBP (5 mg/ml) + 5 µl of distilled water).
4. Add 12.5 µl of Master Mix to every well.
5. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use 1x Kinase Assay Buffer 1 (Diluent Solution).

OR

5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Add 10 µl of 1x Kinase Assay Buffer 1 to the "Blank" wells.
9. Thaw **HPK1 Kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 3 ng/µl with **1x Kinase Assay Buffer 1**.
11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor".

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted HPK1 (3 ng/µl)	-	10 µl	10 µl
Total	25 µl	25 µl	25 µl

12. Incubate at 30°C for 45 minutes.
13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45 minute reaction, add 25 µl of ADP-Glo™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.
17. Add 50 µl of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The “Blank” value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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

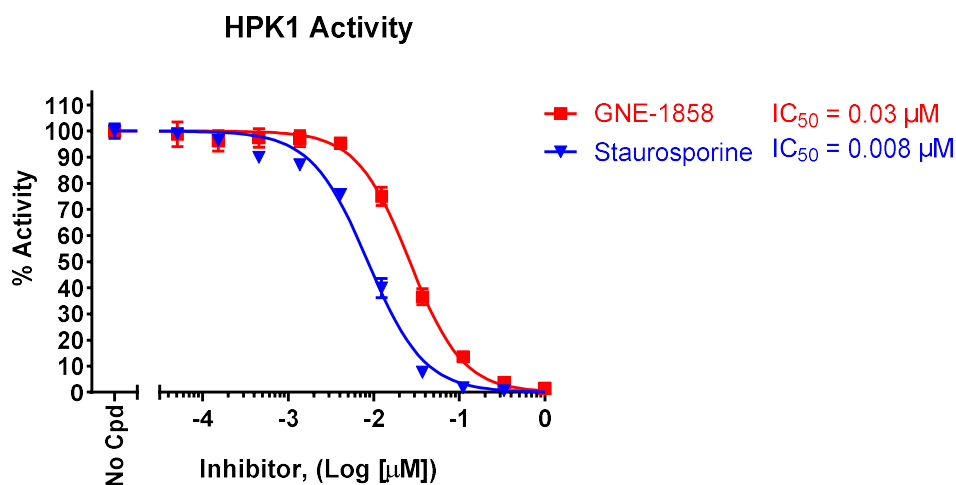


Figure 1: Inhibition of HPK1 kinase activity by the inhibitors GNE-1858 and Staurosporine. HPK1 kinase activity was measured in the presence of increasing concentrations of GNE-1858 (#82585) or Staurosporine (#27002). The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

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

References

Sawadikosol S. and Burakoff S., 2020 *eLife*: 55122.
 You D., et al., 2021 *J Immunother Cancer* 9(1):e001402.
 Noel M., et al., 2024 *Journal of Clinical Oncology* 42(16):suppl

Related Products

Products	Catalog #	Size
HGK (MAP4K4), GST-Tag Recombinant	40109	10 µg
GCK (MAP4K2), GST-Tag Recombinant	40107	10 µg
MAPK4K3 (GLK), GST-Tag Recombinant	100624	5 µg
MINK1, GST-Tag Recombinant	40126	10 µg
Chemi-Verse™ MINK1 Kinase Assay Kit	82580	96 reactions

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