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Data Sheet

NEK6 Kinase Assay Kit

Catalog #79992

Background: NEK6 is a protein kinase in the NIMA-related serine/threonine kinase family that plays a role in mitotic cell cycle progression. Overexpression of NEK6 has been linked to many human diseases including liver, breast, lung, stomach, colon, larynx, ovary and prostate cancer, making NEK6 an attractive target for anti-cancer therapeutics. Inhibition of NEK6 has been shown to induce cell death and premature senescence.

Description: The *NEK6 Kinase Assay Kit* is designed to measure NEK6 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *NEK6 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant NEK6 enzyme, MBP substrate, 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	
40014	NEK6, His-tag	10 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)	50 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

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:
Yin, M.-J., *et al*. 2003. "The serine/threonine kinase Nek6 is required for cell cycle progression through mitosis." *J. Biol. Chem.* **278(52):** 52454-52460.

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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

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

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **MBP (5 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 (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 0.5 µl **MBP (5 mg/ml)** + 8.5 µl water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	3.0 µl	3.0 µl	3.0 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
MBP 5 mg/ml	0.5 µl	0.5 µl	0.5 µl
Distilled water	8.5 µl	8.5 µl	8.5 µl
Test Inhibitor	–	2.5 µl	–
10% DMSO in water (Inhibitor buffer)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
NEK6 (10 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3) Prepare the inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in water (at this step the compound concentration is 10-fold higher than the final concentration in 10% DMSO). To determine an IC50 or to test lower concentrations of the compound, prepare a series of further dilutions in 10% DMSO (aqueous). The final concentration of the DMSO will be 1% in all samples.

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration.

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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 inhibitor buffer (same solution without inhibitor—usually 10% DMSO in water).

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**.

7) Thaw **NEK6** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **NEK6** 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: NEK6 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 **NEK6** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate 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) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “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.

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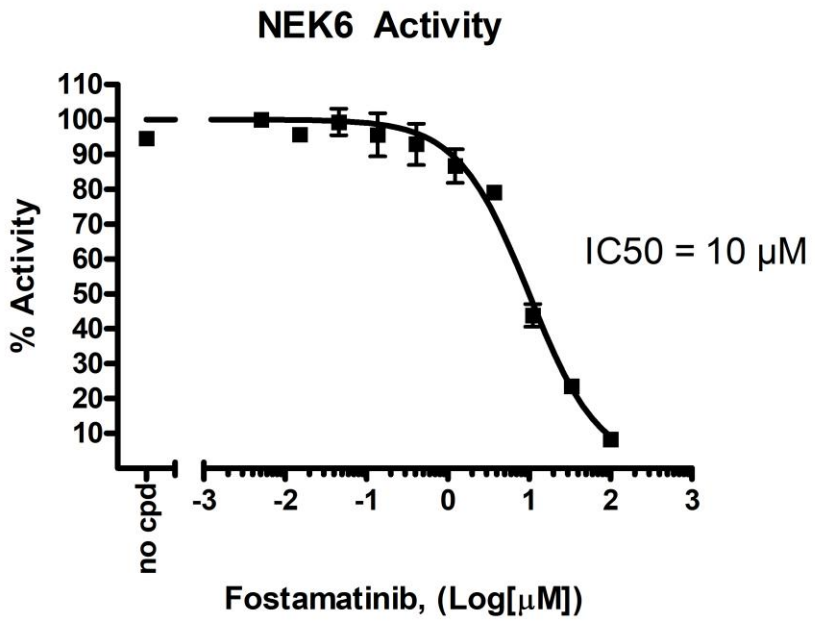
To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position 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).

Example of Assay Results:



Inhibition of NEK6 by Fostamatinib, measured using the *NEK6 kinase assay kit* (BPS Bioscience #79992). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
NEK6, His-tag	40014	10 µg
NEK2, His-tag	40009	10 µg
NEK3, GST-tag	40140	10 µg
NEK7, His-tag	100476	10 µg
NEK7, GST-tag	40141	10 µg
NEK9, GST-tag	40142	10 µg
NEK11, GST-tag	40139	10 µg
5X Kinase assay buffer	79334	10 ml