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Data Sheet

EPHB4 Kinase Assay Kit

Catalog #78009

Background: EPHB4 or Ephrin type-B receptor 4 is a protein and member of the receptor tyrosine kinase family. It binds to its ligand, Ephrin B2, to regulate cell adhesion and migration. EPHB4 plays a central role in heart morphogenesis, angiogenesis and blood vessel remodeling and permeability. It has also been implicated in prostate as well as breast cancer.

Description: The *EPHB4 Kinase Assay Kit* is designed to measure EPHB4 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *EPHB4 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant EPHB4, substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40201	EPHB4, His-tag	20 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	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 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(S):

1. Xia, G., *et al.* 2005. "EphB4 expression and biological significance in prostate cancer." *Cancer Research* **65(11):** 4623-4632.
2. Noren, N.K., *et al.* 2006. "The EphB4 receptor suppresses breast cancer cell tumorigenicity through an Abl–Crk pathway." *Nature Cell Biology* **8(8):** 815-825.

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

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

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 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 (11.5 µl **1x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 0.5 µl **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)**. Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
1x Kinase assay buffer	11.5 µl	11.5 µl	11.5 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor	–	2.5 µl	–
10% DMSO in water (Inhibitor buffer)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
EPHB4 (20 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3) 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 10% DMSO in water (Inhibitor buffer). *Note: Keep DMSO concentration of the Test Inhibitor at ≤10%, as final DMSO concentration in the reaction should 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 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

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.

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

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6) Thaw **EPHB4** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **EPHB4** required for the assay and dilute enzyme to 20 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: EPHB4 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 10 µl of diluted **EPHB4** to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

8) Thaw ADP-Glo reagent.

9) 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.

10) Thaw Kinase Detection reagent.

11) 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.

12) 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.

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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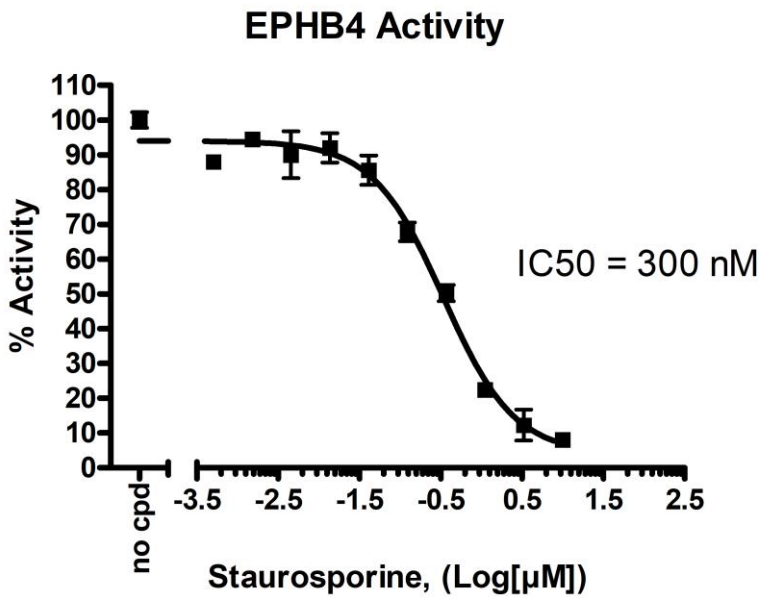
Example of Assay Results:



Inhibition of EPHB4 by Staurosporine, measured using the *EPHB4 kinase assay kit* (Cat. #78009). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
EPHB4, His-tag	40201	10 µg
5x Kinase assay buffer	79334	10 ml
ATP (500 µM)	79686	200 µl
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg
EPHA1, GST-tag	40191	10 µg
EPHA2, His-tag	40190	10 µg
EPHA3, GST-tag	40192	10 µg
EPHA4, GST-tag	40193	10 µg
EPHA6, GST-tag	40194	10 µg
EPHB2, His-tag	40200	10 µg
EPHB3, GST-tag	40186	10 µg
EPHA6, GST-tag	40194	10 µg
EPHB1, GST-tag (Mouse)	40199	10 µg