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Data Sheet

EPHA2 Assay Kit

Catalog #78002
96 Reactions

BACKGROUND: EPHA2 or ephrin type-A receptor 2 is a receptor tyrosine kinase that plays a role in a number of developmental processes, as well as tissue homeostasis and cancer, including prostate cancer. It has also been seen to promote cell motility and cancer metastasis. EphA2 regulation has been linked to lens transparency, kidney repair following renal injury, bone remodeling, and inner ear development.

DESCRIPTION: The *EPHA2 Assay Kit* is designed to measure EPHA2 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *EPHA2 Assay Kit* comes in a convenient 96-well format, with enough purified EPHA2, Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40190	EPHA2, 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	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	RT	

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. Walker-Daniels, J., *et al.* 1999. "Overexpression of the EphA2 tyrosine kinase in prostate cancer." *The Prostate* **41(4):** 275-280.
2. Tandon, M., *et al.* 2011. "Emerging strategies for EphA2 receptor targeting for cancer therapeutics." *Expert opinion on therapeutic targets* **15 (1):** 31-51.

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 0.5 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.

Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**. (Optional: If desired, add 30 µl of 0.5 M DTT to **5x Kinase assay buffer**).

1) Prepare the master mixture (25 µl per well): N wells x (10 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 13 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	10 µl	10 µl	10 µl
ATP (500 µM)	1 µl	1 µl	1 µl
Poly-Glu,Tyr (10 mg/ml)	1 µl	1 µl	1 µl
Water	13 µl	13 µl	13 µl
Test Inhibitor	–	5 µl	–
10% DMSO in water (Inhibitor buffer)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
EPHA2, His-tag (5 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

2) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of 10% DMSO in water (Inhibitor buffer). *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 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

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

5) Thaw **EPHA2, His-Tag** on ice. Upon first thaw, briefly spin tube containing material to recover full content of the tube. Calculate the amount of **EPHA2, His-Tag** required for the assay and dilute enzyme to 5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted material in aliquots at -80°C. *Note: EPHA2, His-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted material.*

6) Initiate reaction by adding 20 µl of diluted **EPHA2, His-Tag** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

7) Thaw Kinase-Glo Max reagent.

8) After the 45 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.

9) Measure luminescence using the microplate reader. Value of “Blank” reading should be subtracted from all other measurements.

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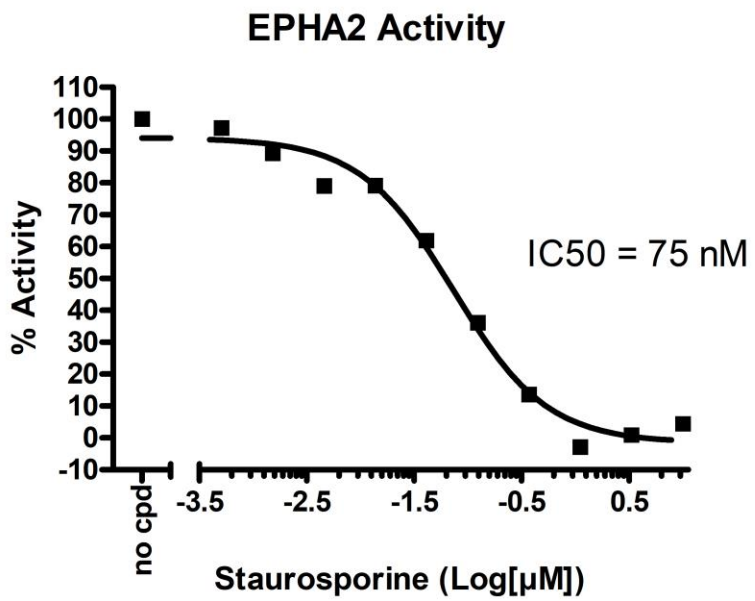
Example of Assay Results:



Inhibition of EPHA2, His-Tag by Staurosporine, measured using the EPHA2 assay kit (BPS Bioscience #78002). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
EPHA2, His-tag	40190	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
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
EPHB4, His-tag	40201	10 µg
EPHA6, GST-tag	40194	10 µg
EPHB1, GST-tag (Mouse)	40199	10 µg