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Data Sheet

VEGFR2(KDR) Kinase Assay Kit

Catalog # 40325

DESCRIPTION: Vascular endothelial growth factor receptor 2 (VEGFR2), also called Kinase insert Domain receptor (KDR), is a tyrosine kinase (TK) receptor for VEGFs that plays a central role in tumor angiogenesis; therefore the inhibition of VEGFR2 is a promising therapeutic strategy for inhibiting angiogenesis and tumor growth. The *VEGFR2 Kinase Assay Kit* is designed to measure VEGFR2 kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *VEGFR2 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant VEGFR2 enzyme, VEGFR2 substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40301	VEGFR2 (KDR)	3 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
	5x Kinase assay buffer	1.5 ml	-20°C	
	ATP (500 µM)	100 µl	-20°C	
40217	50x PTK substrate Poly(Glu:Tyr 4:1)	100 µl	Room Temp.	
	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

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:

Sharma, K., *et al. Biomed Chromatogr.* 2015 Jun; **29(6):**803-34.
Fontanella, C., *et al. Ann Transl Med.* 2014 Dec; **2(12):**123

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

1) Thaw 5x Kinase assay buffer, ATP and 50x PTK substrate.
(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) + 1 µl 50x PTK substrate + 17 µl 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
50x PTK substrate	1 µl	1 µl	1 µl
Water	17 µl	17 µl	17 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
VEGFR2 (~1 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.

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

6) Thaw VEGFR2 enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of VEGFR2 required for the assay and dilute enzyme to 1 ng/µl with 1x Kinase assay buffer. Store remaining undiluted enzyme in aliquots at -80°C. *Note: VEGFR2 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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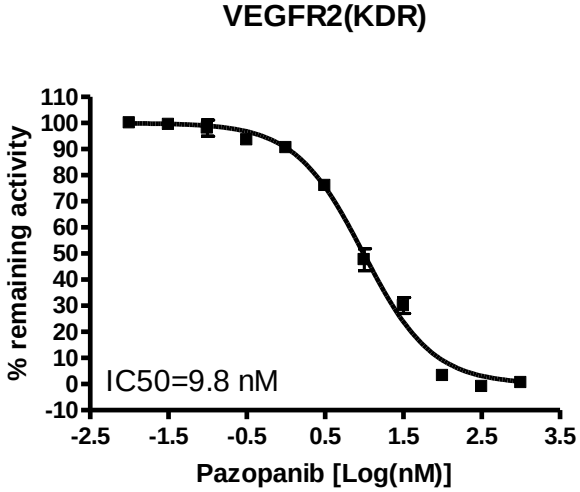
7) Initiate reaction by adding 20 µl of diluted VEGFR2 enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45 minutes, 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.

Example of Assay Results:



Inhibition of VEGFR2 enzyme by Pazopanib, measured using the *VEGFR2(KDR) kinase assay kit* (Cat. #40325). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
VEGFR2 (KDR), GST-tag	40301	10 µg
VEGFR3 (Flt4), GST-tag	40302	10 µg
Flt1 (VEGFR1), His-tag	40223	10 µg
VEGF121, Human (CHO-derived)	91005-1	10 µg
VEGF165, Human (CHO-derived)	91006-1	5 µg
VEGF165, Human (Sf9-derived)	91001-1	10 µg
Rat VEGF-A	91008	10 µg
Mouse VEGF165	91000-1	10 µg
Mouse VEGF 120AA	90253-1	2 µg
Cediranib	27036	5 mg
Axitinib	27064	10 mg
Sorafenib Tosylate	27014	100 mg
EGFR (L858R)	40189	10 µg
EGFR (T790M)	40188	10 µg
EGFR (T790M, L858R)	40350	10 µg
EGFR (T790M, C797S, L858R)	40351	10 µg
EGFR (mouse)	40195	10 µg
EGFR(T790M/L858R) Kinase Assay Kit	40322	384 rxns

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