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Data Sheet

VEGFR3(FLT4) Kinase Assay Kit

Catalog #79738

Background: Vascular endothelial growth factor receptor 3 (VEGFR3), also called Fms-Related Tyrosine Kinase 4 (FLT4), is a tyrosine kinase (TK) receptor for VEGFs that plays a central role in tumor angiogenesis; therefore, the inhibition of VEGFR3 is a promising therapeutic strategy for inhibiting angiogenesis and tumor growth.

Description: The *VEGFR3 Kinase Assay Kit* is designed to measure VEGFR3 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The VEGFR3 *Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant VEGFR3 enzyme, VEGFR3 substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40302	VEGFR3 (FLT4)	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	PTK substrate Poly (Glu:Tyr 4:1) (10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

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

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.

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REFERENCE:

Yeh, Yu-Wen et al. "Targeting the VEGF-C/VEGFR3 axis suppresses Slug-mediated cancer metastasis and stemness via inhibition of KRAS/YAP1 signaling" *Oncotarget* vol. 8,3 (2016): 5603-5618.
Silvia M. Ferrari, et al. "Lenvatinib in the Therapy of Aggressive Thyroid Cancer: State of the Art and New Perspectives with Patents Recently Applied", Recent Patents on Anti-Cancer Drug Discovery (2018) 13: 201.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **PTK substrate (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 (3 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 0.5 µl **50x PTK substrate** + 8.5 µl water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	3 µl	3 µl	3 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
50x PTK substrate	0.5 µl	0.5 µl	0.5 µl
Water	8.5 µl	8.5 µl	8.5 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor Buffer (no inhibitor)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
VEGFR3 (10 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 the same solution without inhibitor (Inhibitor buffer). *Note: Keep DMSO concentration of the Test Inhibitor at ≤10%, as final DMSO concentration in the reaction should be ≤1%.*

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.

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

6) Thaw **VEGFR3** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **VEGFR3** 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: VEGFR3 enzyme 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 **VEGFR3** enzyme 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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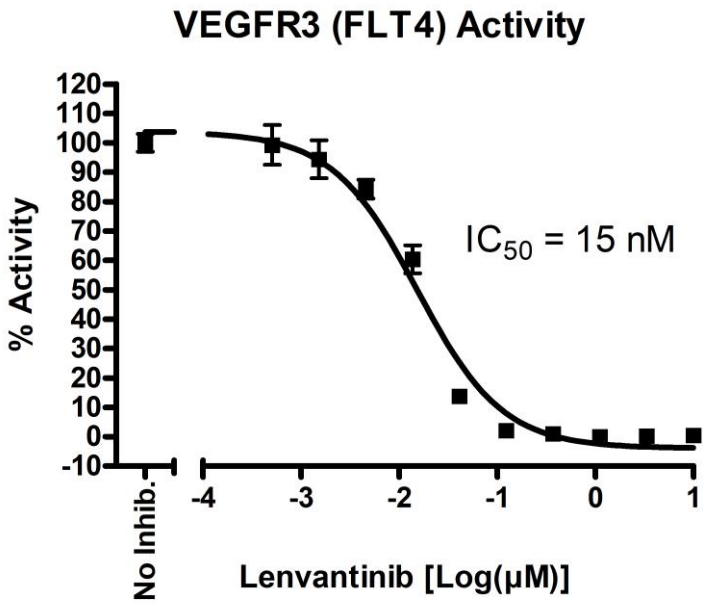
Example of Assay Results:



Inhibition of VEGFR3 enzyme by Lenvantinib, measured using the *VEGFR3 kinase assay kit* (Cat. #79738). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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
5X Kinase assay buffer	79334	10 ml
ATP (500 µM)	79686	200 µl
50x PTK substrate Poly (Glu:Tyr 4:1)	40217	1 mg