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Data Sheet

FGFR3 (V443L) Assay Kit

Catalog #79819
96 Reactions

DESCRIPTION: The *FGFR3 (V443L) Assay Kit* is designed to measure FGFR3 (V443L) activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *FGFR3 (V443L) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant FGFR3 (V443L), Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
100138	FGFR3 (V443L), His-Tag	40 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer 1	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	

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

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:
1. Cappellen, D., *et al*. (1999) "Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas." *Nature Genetics* **23(1):** 18.
2. Billerey, C., *et al.* (2001) "Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors." *Amer. J.Pathology* **158(6):** 1955-1959.

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

1) Thaw **5x Kinase assay buffer 1**, **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**).

2) Prepare the master mixture (25 µl per well): N wells x (10 µl **5x Kinase assay buffer 1**+ 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 1	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	–
I	5 µl	–	5 µl
1x Kinase buffer 1	–	–	20 µl
FGFR3 (V443L), His-tag (20 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 Inhibitor buffer (the same solution without the inhibitor. For compounds dissolved in DMSO, this is usually 10% DMSO in water).
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.

4) Prepare 3 ml of **1x Kinase assay buffer 1** by mixing 600 µl of **5x Kinase assay buffer 1** with 2400 µl water. 3 ml of **1x Kinase assay buffer 1** is sufficient for 100 reactions.

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

6) Thaw **FGFR3 (V443L), His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **FGFR3 (V443L), His-Tag** required for the assay and dilute enzyme to 20 ng/µl

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with **1x Kinase assay buffer 1**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: FGFR3 (V443L), His-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

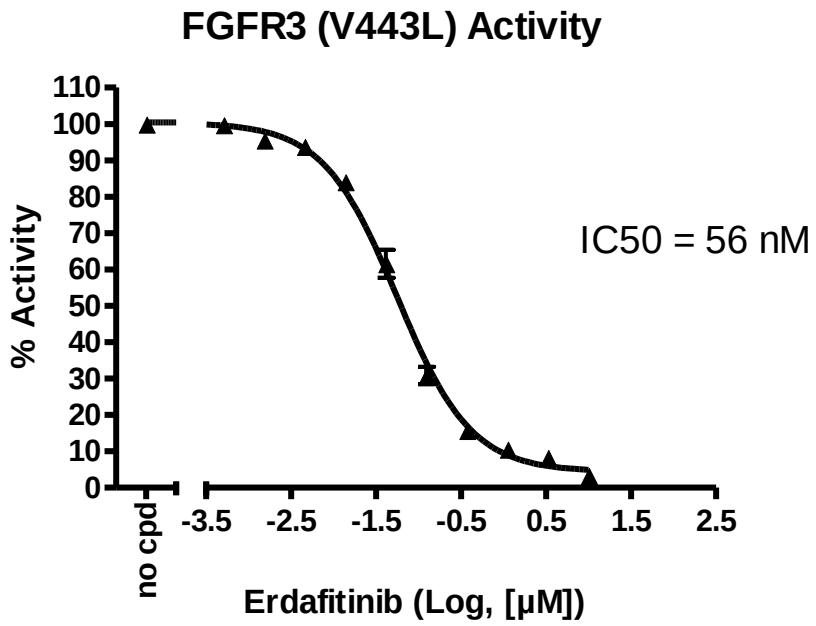
7) Initiate reaction by adding 20 µl of diluted **FGFR3 (V443L), His-Tag** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45 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.

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

Example of Assay Results:



Inhibition of FGFR3 (V443L), His-Tag by Erdafitinib, measured using the FGFR3 (V443L) assay kit (BPS Bioscience #79819). *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
FGFR3 (V443L), His-Tag	100138	10 µg
FGFR3 (V443M), His-Tag	100140	10 µg
FGFR3 (L496V), His-Tag	100142	10 µg
FGFR3 (CD333), GST-tag	40212	10 µg
5x Kinase assay buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg
FGFR1(FLT2), GST-tag	40210	10 µg
FGFR1 (V561M), GST-tag	40209	10 µg
FGFR2, GST-tag	40211	10 µg
FGFR4, GST-tag	40213	10 µg