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Data Sheet
FGFR3 (V443M) Assay Kit
Catalog #79817
96 Reactions

DESCRIPTION: The *FGFR3 (V443M) Assay Kit* is designed to measure FGFR3 (V443M) activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *FGFR3 (V443M) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant FGFR3 (V443M), Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
100140	FGFR3 (V443M), His-Tag	20 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 μ M)**, and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**.
(Optional: If desired, add 30 μ l of 0.5 M DTT to **5x Kinase assay buffer 1**).
- 2) Prepare the master mixture (25 μ l per well): N wells x (10 μ l **5x Kinase assay buffer 1** + 1 μ l **ATP (500 μ M)** + 1 μ l **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 13 μ l distilled water). Add 25 μ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer 1	10 μ l	10 μ l	10 μ l
ATP (500 μ M)	1 μ l	1 μ l	1 μ l
Poly-Glu,Tyr(10 mg/ml)	1 μ l	1 μ l	1 μ l
Water	13 μ l	13 μ l	13 μ l
Test Inhibitor	–	5 μ l	–
Inhibitor buffer	5 μ l	–	5 μ l
1x Kinase buffer 1	–	–	20 μ l
FGFR3 (V443M), His-tag (10 ng/ μ l)	20 μ l	20 μ l	–
Total	50 μl	50 μl	50 μl

- 3) Add 5 μ l of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5 μ l of 10% DMSO in water (Inhibitor buffer).
Note: Final DMSO concentration must be \leq 1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 μ M, dilute 1 mM inhibitor with water to make a 100 μ M inhibitor in 10% DMSO(aq). Then, add 5 μ l of the 100 μ M solution into the 50 μ l 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 μ l of **5x Kinase assay buffer 1** with 2400 μ l water. 3 ml of **1x Kinase assay buffer 1** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank," add 20 μ l of **1x Kinase assay buffer 1**.
- 6) Thaw **FGFR3 (V443M), His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **FGFR3 (V443M), His-Tag** required for the assay and dilute enzyme to 10 ng/ μ l

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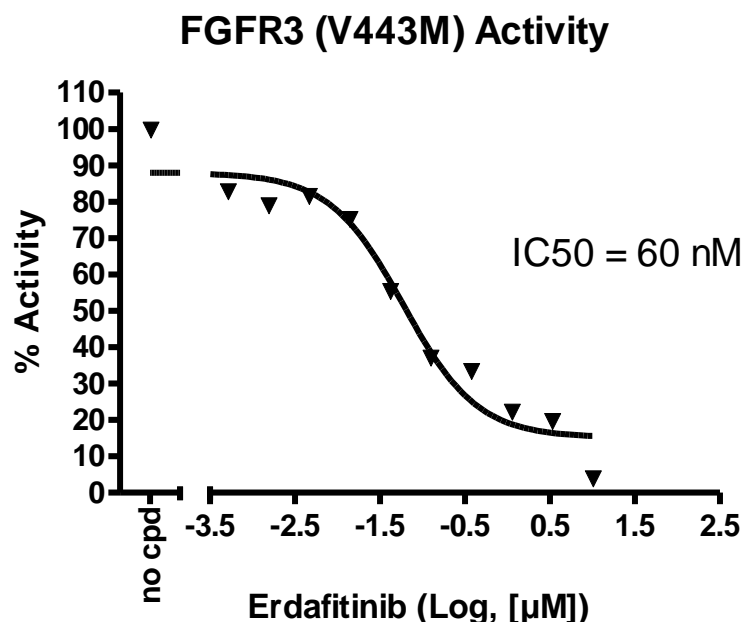


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with **1x Kinase assay buffer 1**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: FGFR3 (V443M), His-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

- 7) Initiate reaction by adding 20 µl of diluted **FGFR3 (V443M), 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 (V443M), His-Tag by Erdafitinib, measured using the FGFR3 (V443M) assay kit (BPS Bioscience #79817). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
FGFR3 (V443M), His-Tag	100140	10 µg
FGFR3 (L496V), His-Tag	100142	10 µg
FGFR3 (V443L), His-Tag	100138	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

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