6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

FGFR2 Assay Kit

Catalog #79804
96 Reactions

BACKGROUND: FGFR2 (Fibroblast Growth Factor Receptor 2) is a member of a family of tyrosine kinases involved in many pathways that play a significant role in cancer. Amplification or activation of FGFR2 has been reported in breast and gastric cancers, while FGFR2 mutations have been observed in endometrial and breast cancers. Mutations in FGFR2 are also associated with bone development disorders including Pfeiffer Syndrome and Crouzon Syndrome.

DESCRIPTION: The *FGFR2 Assay Kit* is designed to measure FGFR2 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *FGFR2 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant FGFR2, Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40211	FGFR2, GST-Tag	5 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	

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.

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REFERENCE:

1. Rutland, P., *et al.* 1995. "Identical mutations in the FGFR2 gene cause both Pfeiffer and Crouzon syndrome phenotypes." *Nature Genetics* **9(2):** 173.
2. Xie, L., *et al*. 2013. "FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547." *Clinical Cancer Research* **9(2):** 2572-2583.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) 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**).

2) 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
FGFR2, GST-tag (2.5 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), 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.

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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 **FGFR2, GST-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **FGFR2, GST-Tag** 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: FGFR2, GST-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 **FGFR2, GST-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.

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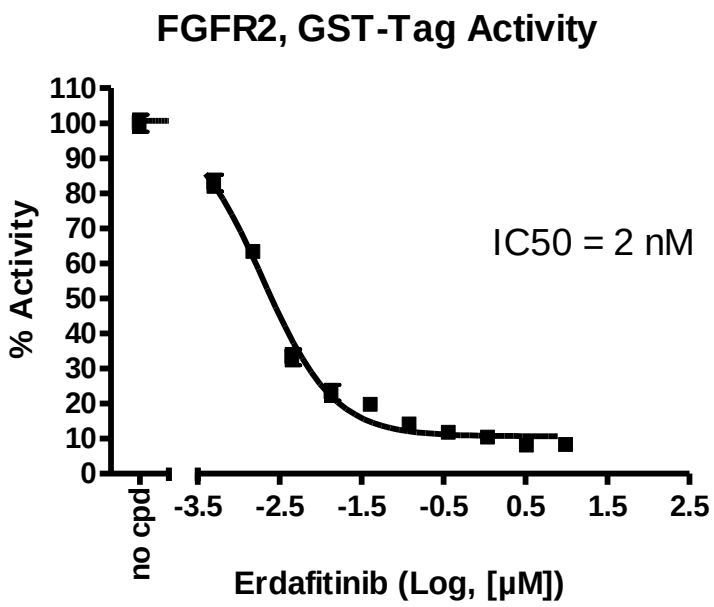
Example of Assay Results:



Inhibition of FGFR2, GST-Tag by Erdafitinib, measured using the FGFR2 assay kit (BPS Bioscience #79804). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
FGFR2, GST-tag	40211	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
FGFR1(FLT2), GST-tag	40210	10 µg
FGFR1 (V561M), GST-tag	40209	10 µg
FGFR3 (CD333), GST-tag	40212	10 µg
FGFR3 (V443M), His-Tag	100140	10 µg
FGFR3 (L496V), His-Tag	100142	10 µg
FGFR3 (V443L), His-Tag	100138	10 µg
FGFR4, GST-tag	40213	10 µg
FGFR1(FLT2), GST-tag	40210	10 µg
FGFR1(FLT2), GST-tag	40210	10 µg
FGFR1(FLT2), GST-tag	40210	10 µg
FGFR1(FLT2), GST-tag	40210	10 µg