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Data Sheet
PDGFR α (D842Y) Assay Kit
Catalog #79760
96 Reactions

DESCRIPTION: Platelet-derived growth factor receptor A or PDGFR α has been implicated in regulation of cell growth and survival, apoptosis, and differentiation. It has been identified as a potential target in eosinophilic leukemia cancer, inflammatory breast cancer, and gastrointestinal stromal tumors. The D842 mutation is found in gastrointestinal stromal tumors and mutations at this site, other than D842Y, are associated with resistance to tyrosine kinase inhibitors. The *PDGFR α (D842Y) Assay Kit* is designed to measure PDGFR α (D842Y) activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The *PDGFR α (D842Y) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant PDGFR α (D842Y), Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
100201	PDGFR α (D842Y), GST-Tag	10 μ g	-80°C	Avoid multiple freeze/thaw cycles!
79334	5x Kinase Assay Buffer 1	1.5 ml	-20°C	
79686	ATP (500 μ M)	100 μ l	-20°C	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 μ l	-20°C	
79696	96-well plate, white	1	RT	

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:

1. Evans, E., *et al.* A Precision Therapy Against Cancers Driven by KIT/PDGFR α Mutations; 2017, *Science Translational Medicine* Nov; **9(414)**: 1690.

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2. Joglekar-Javadekar, M., *et al.* Characterization and Targeting of Platelet-Derived Growth Factor Receptor alpha (PDGFRA) in Inflammatory Breast Cancer (IBC). *Neoplasia*. 2017 Jul; **19(7)**:564-573.
3. Corless, C.L., *et al.* PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. *J Clin Oncol*. 2005 Aug 10; **23(23)**:5357-64.

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 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 (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
PDGFRα (D842Y) (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 10% DMSO in water (Inhibitor buffer). 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. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity.*
- 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 20 μ l of **1x Kinase assay buffer**.
- 6) Thaw **PDGFR α (D842Y)**, **GST-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **PDGFR α (D842Y)**, **GST-Tag** required for the assay and dilute enzyme to 5 ng/ μ l with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PDGFR α (D842Y), 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 μ l of diluted **PDGFR α (D842Y)**, **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 μ l 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. "Blank" value is subtracted from all readings.

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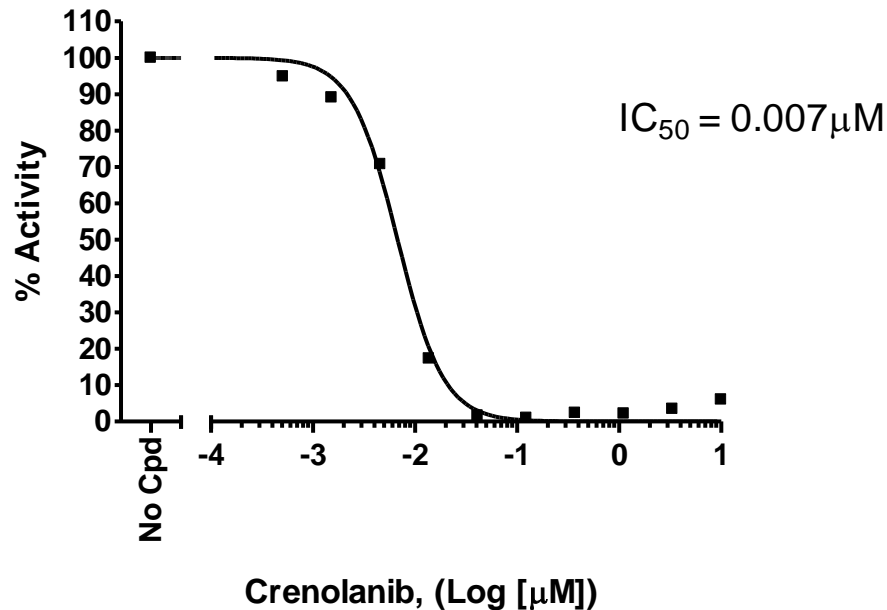
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Example of Assay Results:

PDGFR α (D842Y) Activity



Inhibition of PDGFR α (D842Y), GST-Tag by Crenolanib, measured using the PDGFR α (D842Y) assay kit (BPS Bioscience #79760). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
PDGFR α (D842Y), GST-Tag	#100201	10 μg
PDGFR α (D842I), GST-Tag	#100202	10 μg
PDGFR α (D842V), GST-Tag	#79633	10 μg
PDGFR α , GST-Tag	#40261	10 μg
PDGFR α , Mouse, GST-Tag	#40260	10 μg
PDGFR α (D842I) Assay Kit	#xxxxx	96 rxns.
PDGFR β , His-tag	#40263	10 μg

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