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Data Sheet

FLT3 Kinase Assay Kit

Catalog #79797
Size : 96 reactions

Description: The *FLT3 Kinase Assay Kit* is designed to measure FLT3 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The FLT3 *Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant FLT3 enzyme, substrate, ATP, and kinase assay buffer for 100 enzyme reactions.
MBP (Myelin Basic Protein) is a non-specific protein substrate that is used as a "universal substrate" for many in-vitro kinase activity assays. This protein is targeted by many serine/threonine kinases at conserved amino acids. We use the dephosphorylated version of the MBP substrate in our assays to determine the kinase-mediated phosphorylation of MBP. Our assays are not suitable for studying autophosphorylation of the kinase due to the presence of the MBP substrate.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40225	FLT3	2 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	
	Myelin basic protein (MBP) (5 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:

1. Swords, R., Freeman, C., and Giles, F. 2012. "Targeting the FMS-like tyrosine kinase 3 in acute myeloid leukemia." *Leukemia* **26(10):** 2176-2185.
2. Parcells, B.W., *et al.* 2006. "FMS-like tyrosine kinase 3 in normal hematopoiesis and acute myeloid leukemia." *Stem cells* **24(5):** 1174-1184.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **MBP (5 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 (10 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 1 µl **MBP (5 mg/ml)** + 5.5 µl water). Add 10 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
MBP (5 mg/ml)	1 µl	1 µl	1 µl
Water	5.5 µl	5.5 µl	5.5 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor Buffer (no inhibitor)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	12.5 µl
FLT3 (3.0 ng/µl)	12.5 µl	12.5 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 2,400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

5) To the wells designated as "Blank," add 12.5 µl of **1x Kinase assay buffer**.

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6) Thaw **FLT3** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **FLT3** required for the assay and dilute enzyme to 3 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: FLT3 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 12.5 µl of diluted **FLT3** 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).

Example of Assay Results:

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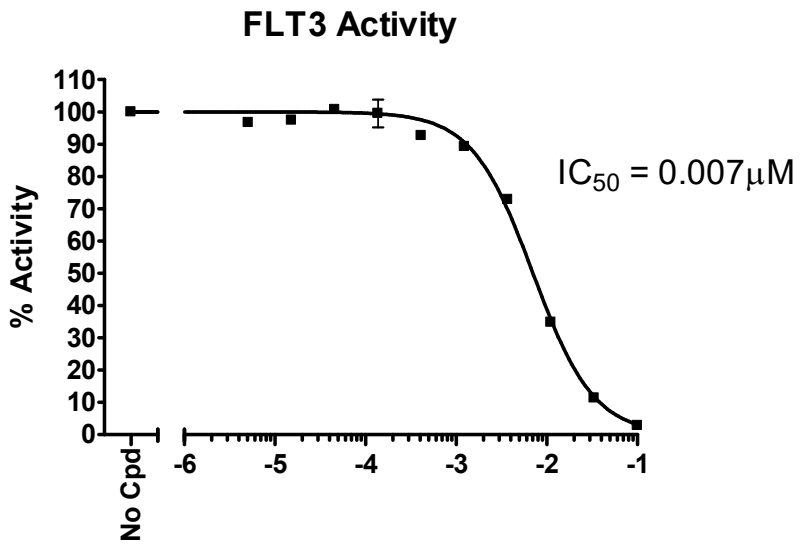

Inhibition of FLT3 enzyme by Giltertinib, measured using the *FLT3 kinase assay kit* (BPS Bioscience #79797). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
FLT3, His-tag	40225	10 µg
Kinase Buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl
Mouse FLT3 Ligand	90136-B	10 µg
Mouse FLT3 Ligand	90136-B	10 µg
FLT1, His-tag	40223	10 µg
FGFR1(FLT2), GST-tag	40210	10 µg
FGFR1 (V561M), GST-tag	40209	10 µg
VEGFR3 (FLT4), GST-tag	40302	10 µg
VEGFR3(FLT4) Kinase Assay Kit	79738	96 rxns.