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Data Sheet

FLT1 Kinase Assay Kit

Catalog #78019

Background: FLT1 is a protein kinase in the src gene family that plays a role in mitotic cell cycle progression. Alteration of FLT1 has been shown in various cancers such as breast carcinoma and melanoma.

Description: The *FLT1 Kinase Assay Kit* is designed to measure FLT1 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *FLT1 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant FLT1 enzyme, Poly-(Glu,Tyr 4:1), ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40223	FLT1	10 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	Poly-(Glu,Tyr 4:1) (10 mg/ml)	50 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.

REFERENCE:

1. Autiero, M., *et al.* 2003. "Role of PlGF in the intra-and intermolecular cross talk between the VEGF receptors Flt1 and Flk1." *Nature Medicine* **9(7):** 936-943.
2. Kaipainen, A., *et al.* 1993. "The related FLT4, FLT1, and KDR receptor tyrosine kinases show distinct expression patterns in human fetal endothelial cells." *The Journal of Experimental Medicine* **178(6):** 2077-2088.

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

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Poly-(Glu,Tyr 4:1) (10 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 (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 0.5 µl **Poly-(Glu,Tyr 4:1) (10 mg/ml)** + 8.5 µl water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
1x Kinase assay buffer	11.5 µl	11.5 µl	11.5 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
Poly-(Glu,Tyr 4:1) 10 mg/ml	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor	–	2.5 µl	–
10% DMSO in water (Inhibitor buffer)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
FLT1 (10 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3) Prepare the inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in water (at this step the compound concentration is 10-fold higher than the final concentration in 10% DMSO). To determine an IC50 or to test lower concentrations of the compound, prepare a series of further dilutions in 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

4) 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 Inhibitor buffer (same solution without inhibitor, usually 10% DMSO in water).

5) 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.

6) To the wells designated as "Blank," add 10 µl of **1x Kinase assay buffer**.

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7) Thaw **FLT1** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **FLT1** required for the assay and dilute enzyme to 10 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: FLT1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

8) Initiate reaction by adding 10 µl of diluted **FLT1** enzyme to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

9) Thaw ADP-Glo reagent.

10) 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.

11) Thaw Kinase Detection reagent.

12) 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.

13) 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).

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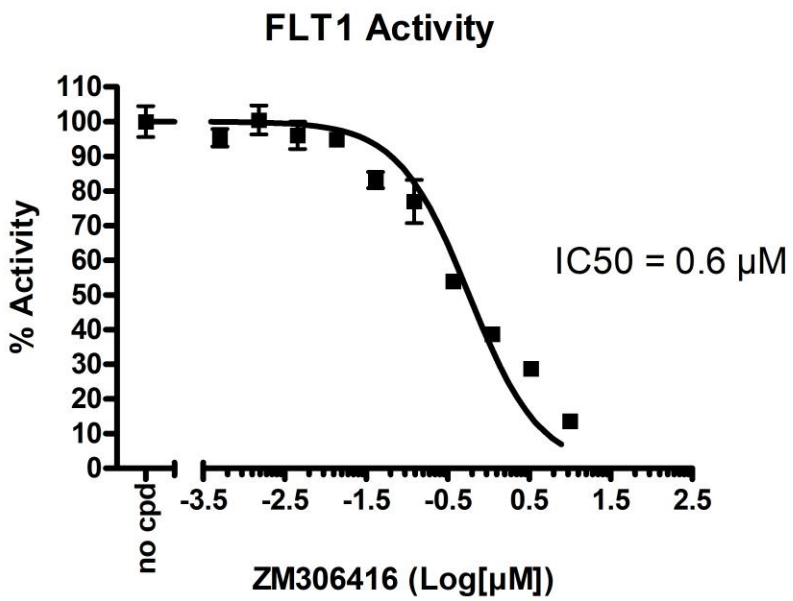
Example of Assay Results:



Inhibition of FLT1 by ZM306416 (Selleck Chem #S2897), measured using the *FLT1 kinase assay kit* (BPS Bioscience #78019). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
FLT1, His-tag	40223	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
FLT3, His-tag	40225	10 µg
VEGFR3(FLT4), GST-tag	40225	10 µg
FGFR1 (V561M), GST-tag	40209	10 µg
FLT3 Kinase Assay Kit	79797	96 rxns.
VEGFR3(FLT4) Kinase Assay Kit	79738	96 rxns.