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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

cKIT (D816V) Assay Kit

Catalog #79889
96 Reactions

Background: c-KIT is a proto-oncogene and a type III transmembrane receptor for mast cell growth factor, also known as stem cell factor. It plays an essential role in the regulation of cell survival and proliferation, as well as hematopoiesis, stem cell maintenance, gametogenesis, melanogenesis, and mast cell development, migration and function. The D816V substitution in the cKIT kinase domain has been identified in peripheral blood mononuclear cells of patients suffering from mastocytosis with an associated hematologic disorder. Gastrointestinal stromal tumors (GIST), acute myeloid leukemia, and germ cell tumors have also been demonstrated to carry this mutation.

DESCRIPTION: The *cKIT (D816V) Assay Kit* is designed to measure cKIT (D816V) activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *cKIT (D816V) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant cKIT (D816V) enzyme, Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40252	cKIT (D816V), His-Tag	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	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

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.

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STABILITY: Up to 6 months when stored as recommended.

REFERENCES:

1. Schumaker, J.A., *et al.* (2008) *J. Clin. Pathol.* **61:**109-114.

2. Sun, J. *et al.* (2009) *J. Biol. Chem.* **284:** 11039-11047.

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 (6 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 17 µl distilled. Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
Protein Tyrosine Kinase Substrat (Poly-Glu,Tyr 4:1)	1 µl	1 µl	1 µl
Water	17 µl	17 µl	17 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
cKIT (D816V) (1 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Prepare the inhibitor solution.

The final concentration of DMSO in the assay should not exceed 1%. If the test 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 1X assay buffer (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

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series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer.

4) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” Add 5 µl of inhibitor buffer (1X assay buffer or 10% DMSO, depending which inhibitor solution is used) to “Blank” and “Positive Control” wells.

5) Prepare 2 ml of **1x Kinase assay buffer** by mixing 400 µl of **5x Kinase assay buffer** with 1600 µl distilled water. 2 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

6) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

7) Thaw **cKIT (D816V)** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **cKIT (D816V)** 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: cKIT (D816V) 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 20 µl of diluted **cKIT (D816V)** to the wells designated “Positive Control” and "Test Inhibitor" Incubate at 30˚C for 45 minutes.

9) Thaw Kinase-Glo Max reagent.

10) 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.

11) Measure luminescence using the microplate reader. “Blank” value should be subtracted from all wells.

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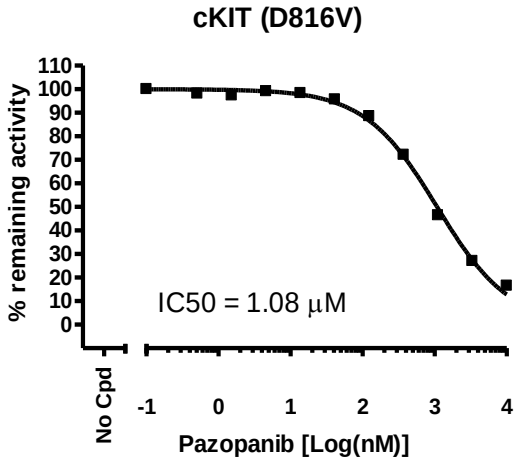
Example of Assay Results:



Inhibition of cKIT (D816V) by Pazopanib and Imatinib, measured using the cKIT (D816V) assay kit (BPS Bioscience #79889). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
cKIT (wild type), His-tag	40250	10 µg
cKIT (D816V), His-tag	40252	10 µg
cKIT (V560G), His-tag	40253	10 µg
cKIT (V654A), His-tag	40251	10 µg
5x Kinase buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg