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

cKIT Assay Kit

Catalog #79815
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. Activating mutations in cKIT are associated with gastrointestinal stromal tumors, testicular seminoma, mast cell disease, melanoma, and acute myeloid leukemia, while inactivating mutations are associated with the genetic defect piebaldism.

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

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40250	cKIT, His-Tag	20 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase Assay Buffer 1	1.5 ml	-20°C	
79686	ATP (500 µM)	250 µl	-20°C	
40217	Protein Tyrosine Kinase Substrate (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.

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REFERENCES:

1. Picardo, M., Cardinali, G. *J Invest Dermatol.* 2011 Jun;**131(6):**1182-5.

2. Yarden, Y., *et al. EMBO J*. 1987; **6 (11):**3341-51.

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 (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 2.5 µl **ATP (500 µM)** + 0.5 µl **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 6.5 µl distilled. Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	3 µl	3 µl	3 µl
ATP (500 µM)	2.5 µl	2.5 µl	2.5 µl
Protein Tyrosine Kinase Substrat (Poly-Glu,Tyr 4:1)	0.5 µl	0.5 µl	0.5 µl
Water	6.5 µl	6.5 µl	6.5 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor Buffer (no inhibitor)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
cKIT (18 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 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 2.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” Add 2.5 µl of inhibitor buffer (1X assay buffer or 10% DMSO, depending on 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 10 µl of **1x Kinase assay buffer**.

7) Thaw **cKIT** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **cKIT** required for the assay and dilute enzyme to 18 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C.

Note: cKIT 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 **cKIT** 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 30 minutes.

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

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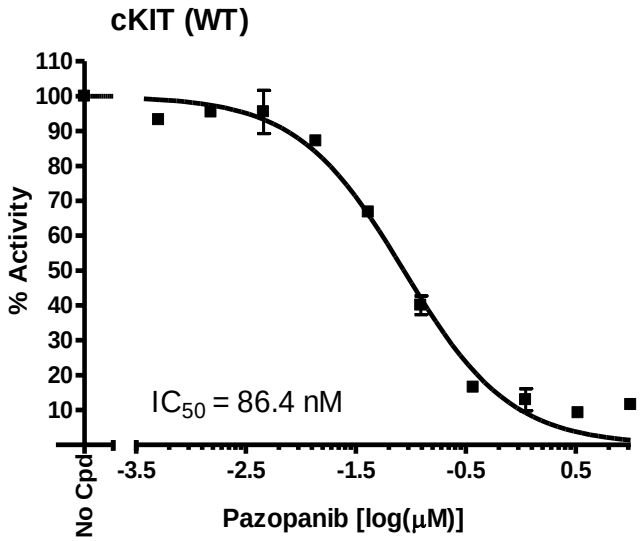
Example of Assay Results:



Inhibition of cKIT by Pazopanib, measured using the cKIT assay kit (BPS Bioscience #79815). *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