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Data Sheet
RET Assay Kit
Catalog #79566

DESCRIPTION: RET is a receptor kinase for GDNF (glial cell-line derived neurotrophic factor) family ligands (GFLs). Activation of wild-type RET is important in several cancers where it contributes to tumor progression through various mechanisms. In addition, it has been shown that activating mutation as well as RET rearrangement that leads constitutively active protein plays a significant role in various cancer types. Importantly, small molecule inhibitors of RET have been clinically proved as a promising therapeutic reagent in medullary thyroid cancer and are being evaluated for other types of cancers related to RET overexpression or mutation. The *RET Assay Kit* is designed to measure RET activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *RET Assay Kit* comes in a convenient 96-well format, with enough purified recombinant RET enzyme, RET substrate peptide (IRF-1Rtide), ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40267	RET	15 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer 1	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
79567	IGF-1Rtide (1 mg/ml)	500 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.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE:
Mulligan, L. M., *Nature Reviews Cancer* **14:**173-186 (2014)

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

1) Thaw **5x Kinase assay buffer 1**, **ATP** and **IGF-1Rtide (1 mg/ml)**. (Optional: If desired, add DTT to **5x Kinase assay 1 buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer 1**)

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer 1** + 1 µl **ATP (500 µM)** + 5 µl **IGF-1Rtide (1 mg/ml)** + 13 µl water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer 1	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
IGF-1Rtide (1 mg/ml)	5 µl	5 µl	5 µl
Water	13 µl	13 µl	13 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer 1	–	–	20 µl
RET (7.5 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 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 1** by mixing 600 µl of **5x Kinase assay buffer 1** with 2400 µl water. 3 ml of **1x Kinase assay buffer 1** is sufficient for 100 reactions.

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

6) Thaw **RET enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **RET** required for the assay and dilute enzyme to ~7.5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **RET** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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7) Initiate reaction by adding 20 µl of **diluted RET enzyme** to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30˚C for 60 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 60 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.

10) 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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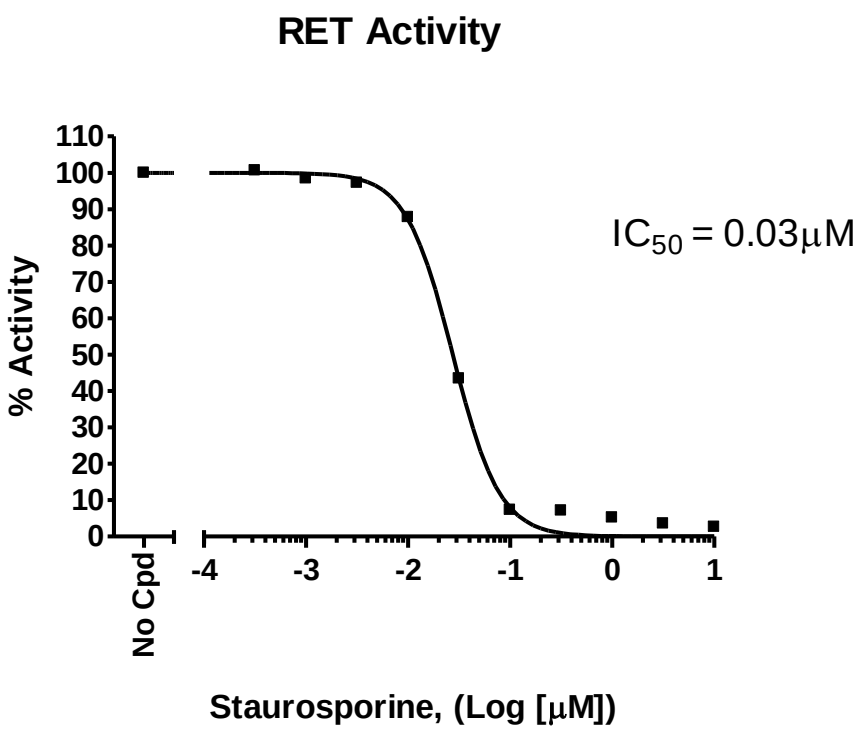
Example of Assay Results:



Inhibition of RET enzyme by Staurosporine (BPS Bioscience #27002), measured using the RET assay kit (BPS Bioscience #79566). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
Ret, GST-tag	40267	10 µg
IGF-1R, GST-tag	40240	10 µg
Staurosporine	27002	10 mg
5x Kinase assay buffer	79334	10 ml