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Data Sheet
TrkA Assay Kit
Catalog #79548

DESCRIPTION: The tropomyosin receptor kinases (TRK) are a family of tyrosine receptor kinases, which include TrkA, TrkB and TrkC. TrkA is activated by binding to nerve growth factor (NGF), resulting in activation of cell proliferation through the RAS/MAPK/ERK and PLCγ/PI3K pathways. Constitutive activation of TrkA is associated with colorectal cancer, suggesting TrkA inhibitors may have potential therapeutic value. The *TrkA Assay Kit* is designed to measure TrkA activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *TrkA Assay Kit* comes in a convenient 96-well format, with enough purified recombinant TrkA enzyme, TrkA substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40280	TrkA	10 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	
40217	PTK 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.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE: Lange, A.M. et. al., *Cancer(Basel)* **10(4):**105 (2018)

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

1) Thaw **5x Kinase Assay Buffer 1**, **ATP**, and **PTK Substrate Poly (Glu:Tyr 4:1) (10 mg/ml)**.
(Optional: If desired, add DTT to **5x Kinase Assay Buffer 1** 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)** + 1 µl **PTK Substrate Poly (Glu:Tyr 4:1) (10 mg/ml)** + 17 µl water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase buffer 1	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
PTK substrate (10 mg/ml)	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
TrkA (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 is sufficient for 100 reactions.

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

6) Thaw **TrkA** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **TrkA** required for the assay and dilute enzyme to 5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **TrkA** 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 **TrkA** enzyme to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) 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.

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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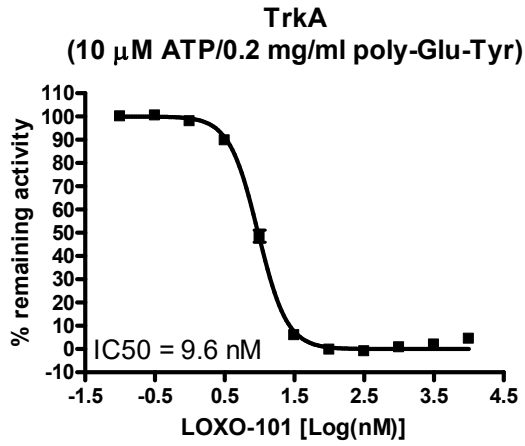
Example of Assay Results:



Inhibition of TrkA enzyme by LOXO-101, measured using the TrkA kinase assay kit (Cat. #79548). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
TRKA, GST-tag	40280	10 µg
TRKB, GST-tag	40281	10 µg
TRKC, GST-tag	40282	10 µg
TRKC (G623E) Mutant, Active)	40204	10 µg
TRKC (G623R) Mutant, Active)	40203	10 µg
TRKC (L686M), Mutant, Active	40216	10 µg
TRKC (G623R L686M),Mutant, Active	40215	10 µg

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