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Data Sheet ***TYRO3 Kinase Assay Kit*** **Catalog # 79593**

DESCRIPTION: TYRO3 is a member of the TAM receptor kinases and plays an important role in cell proliferation/survival, adhesion/migration, and regulation of inflammatory cytokine release. Along with other members of this family, Axl and c-Mer (MERTK), TYRO3 is part of an emerging class of innate immune checkpoints participating in anti-tumoral immunity. Thus, it has been suggested that TYRO3 inhibition could be a promising immunotherapeutic approach in cancer treatment. The *TYRO3 Assay Kit* is designed to measure TYRO3 activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The *TYRO3 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant TYRO3 enzyme, TYRO3 substrate peptide, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40293	TYRO3	4 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:

Akalu, Y.T., *et. al. Immunological Reviews* **276**:165-177 (2017)

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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 (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 (10 mg/ml)** + 17 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
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 1	–	–	20 µl
TYRO3 (1.5 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).
- 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 **TYRO3 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **TYRO3** required for the assay and dilute enzyme to ~1.5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: TYRO3 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

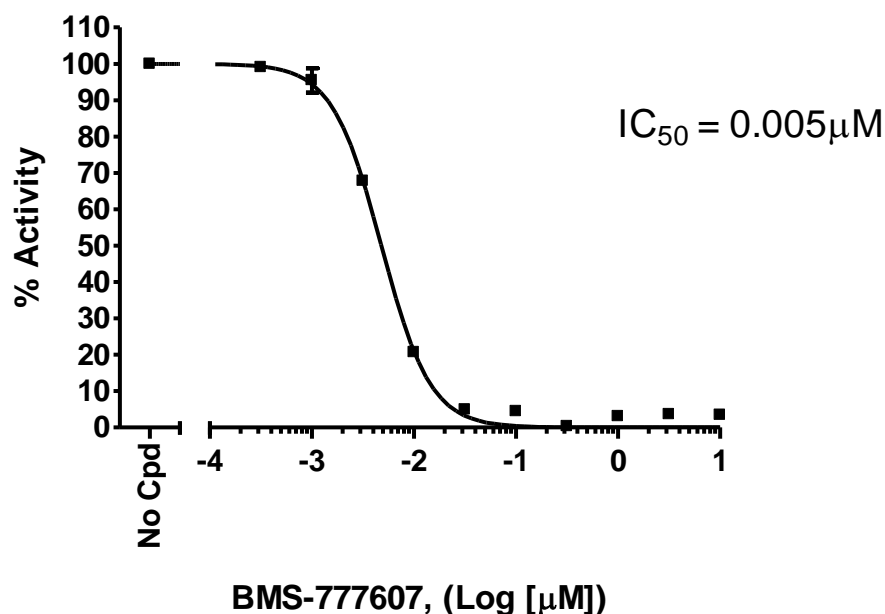
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- 7) Initiate reaction by adding 20 μ l of **diluted TYRO3 enzyme** to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50 μ l of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
- 10) Measure luminescence using the microplate reader. "Blank" value is subtracted from all readings.

Example of Assay Results:**TYRO3 Activity**

Inhibition of TYRO3 enzyme by BMS-777607, measured using the TYRO3 assay kit (Cat. #79593). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
TYRO3, GST-tag	40293	10 µg
c-Mer, GST-tag (Human)	40254	10 µg
Axl, GST-tag	40180	10 µg
GAS Reporter (Luc) – HeLa Cell Line	79041	2 vials
Kinase Buffer 1	79334	10 ml
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg

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