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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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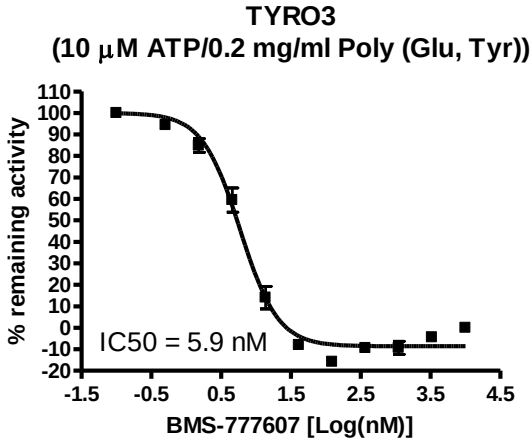
7) Initiate reaction by adding 20 µl of **diluted TYRO3 enzyme** to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45 minute reaction, add 50 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) Measure luminescence using the microplate reader. “Blank” value is subtracted from all readings.

Example of Assay Results:



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 info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
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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