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Data Sheet ***c-Mer Kinase Assay Kit*** **Catalog #79660**

DESCRIPTION: c-Mer (MERTK) 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 TYRO3, c-Mer is part of an emerging class of innate immune checkpoints participating in anti-tumoral immunity. Thus, it has been suggested that c-Mer inhibition could be a promising immunotherapeutic approach in cancer treatment. The *c-Mer Kinase Assay Kit* is designed to measure c-Mer activity for screening and profiling applications using ADP-Glo® as a detection reagent. The *c-Mer Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant c-Mer enzyme, c-Mer substrate peptide, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40254	c-Mer	6 µg	-80°C	<i>Avoid multiple freeze/thaw cycles!</i>
79334	5x Kinase assay buffer 1	1.5 ml	-20°C	
	ATP (500 µM)	100 µl	-20°C	
40217	50x PTK substrate, Poly (Glu:Tyr, 4:1)	100 µl	Room Temp.	
	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

ADP-Glo (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.

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 **50x PTK substrate**.
 (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 (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer 1** + 0.5 µl **ATP (500 µM)** + 0.5 µl **50x PTK substrate** + 8.5 µl water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer 1	3 µl	3 µl	3 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
50x PTK substrate	0.5 µl	0.5 µl	0.5 µl
Water	8.5 µl	8.5 µl	8.5 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor Buffer (no inhibitor)	2.5 µl	–	2.5 µl
1x Kinase buffer 1	–	–	10 µl
cMer (5 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

- 3) Add 2.5 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 2.5 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 10 µl of **1x Kinase assay buffer**.
- 6) Thaw **cMer enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **cMer** 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: cMer enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 7) Initiate reaction by adding 10 µl of **diluted cMer enzyme** to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30°C for 45 minutes.

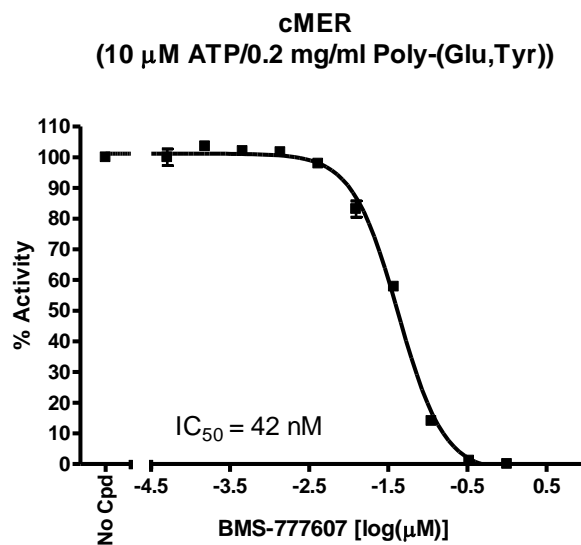
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- 8) Thaw ADP-Glo reagent.
- 9) After the 45 minute reaction, add 25 μ l of ADP-Glo reagent to each well and incubate the plate at room temperature for 45 min.
- 10) Add 50 μ m of Kinase-Detection reagent. Cover the plate with aluminum foil and incubate it at room temperature for another 45 minutes.
- 11) Measure luminescence using the microplate reader. "Blank" value is subtracted from all readings.

Example of Assay Results:

Inhibition of cMer enzyme by BMS777607, measured using the cMer Kinase Assay kit (Cat. #79660). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@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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