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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

c-Met Exon 14 Del Kinase Assay Kit

Catalog # 79559
Size : 96 reactions

Background: c-Met, also known as HGFR (hepatocyte growth factor receptor), is a receptor protein tyrosine kinase encoded by the gene *MET*. Upon binding its ligand HGF (hepatocyte growth factor), c-Met activates multiple cellular processes including proliferation, adhesion and angiogenesis. Importantly, many studies report that c-Met is overexpressed in various carcinomas, suggesting that targeting HGF/c-Met signaling could be a promising target for cancer treatment. A splice mutation that results in skipping exon 14 has been identified in ~4% of lung cancer patients, particularly those with non small cell lung cancer. This mutation causes over-expression of MET protein and increased MET activation, leading to oncogenesis.
Description: The *c-Met Exon 14 Del Kinase Assay Kit* is designed to measure c-Met exon 14 deletion kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *c-Met Exon Del 14 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant c-Met Exon 14 Del enzyme, c-Met substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40255	c-Met Exon 14 Del	2.5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	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.

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REFERENCE:

Awad, M.M., et al. "MET exon 14 mutations in non–small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and c-Met overexpression." *J. Clin. Oncology* (2016). **34 (7):** 721-30

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP** and **PTK substrate Poly (Glu:Tyr, 4:1) (10 mg/ml)**.
   (Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer**)

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer** + 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 assay buffer	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 (10% DMSO in water)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
c-Met Exon 14 del (~0.8 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Prepare 10X concentrated inhibitor in an aqueous-based solution. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution to the assay to make a 1% DMSO concentration in the final reaction mixture*

4) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of 10% DMSO in water (Inhibitor buffer).

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Note: Keep DMSO concentration of the Test Inhibitor at ≤10%, as final DMSO concentration in the reaction should be ≤1%.

5) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

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

7) Thaw **c-Met Exon 14 del enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **c-Met Exon 14 del** required for the assay and dilute enzyme to 0.8 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **c-Met Exon 14 del** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

8) Initiate reaction by adding 20 µl of **diluted c-Met Exon 14 del enzyme** to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

9) Thaw Kinase-Glo Max reagent.

10) 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.

11) Immediately read sample in a luminometer or microtiter-plate capable of reading luminescence. “Blank” value is subtracted from all readings.

Reading Luminescence:

Luminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read luminescence, 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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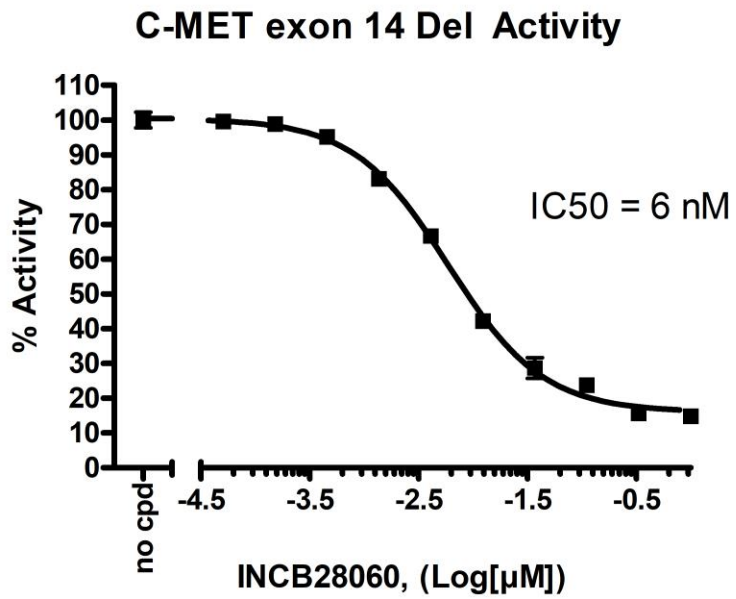
Example of Assay Results:



Inhibition of c-Met del 14 enzyme by INCB28060 measured using the *c-Met 14 Del Kinase Assay Kit* (Cat. #79559). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
Human cMet, GST-tag	40255	10 µg
Human c-MET (del 963-1009), GST-tag	100643	10 µg
Human c-Met 14 Del	xxxxx	10 µg
Rat Met, GST-tag	40228	10 µg
Human Hepatocyte Growth Factor α chain	90157-A	2 µg
Human Hepatocyte Growth Factor α chain	90157-B	10 µg
c-Met Kinase Assay Kit	79559	96 rxns.