Description

The c-Met Kinase Assay Kit is designed to measure c-Met kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant c-Met kinase, kinase substrate, ATP and kinase assay buffer for 100 enzyme reactions. The recombinant protein used in the kit corresponds to amino acids 956-1390 of c-Met, containing the tyrosine kinase domain.

Background

c-Met, also known as HGFR (hepatocyte growth factor receptor), is a tyrosine kinase receptor 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, c-Met is overexpressed in various carcinomas, suggesting that HGF/c-Met signaling pathway could be a promising target for cancer treatment.

Applications

1. Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40255	c-Met, GST-tag*	2.5 µg	-80°C
79334	5x Kinase Assay Buffer	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
40217	Substrate Poly (Glu:Tyr, 4:1) (10 mg/ml)	100 µl	-20°C
79696	White 96-well plate	1	Room Temperature

The concentration of the protein is lot-specific and will be indicated on the tube

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo MAX	Promega #V6071
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

Kinase activity is measured using Kinase-Glo™ Max (Promega; #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100 µM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

All samples and controls should be tested in duplicate.

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

2. Prepare the Master Mix (25 µl/well): N wells x (6 µl of **5x Kinase assay buffer** + 1 µl of **ATP (500 µM)** + 1 µl of **Poly (Glu:Tyr, 4:1) (10 mg/ml)**+ 17 µl of distilled water). Add 25 µl to every well.

3. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use distilled water (Diluent Solution).

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate solution. The concentration of DMSO is now 10%.
   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor.” For the “Positive Control" and “Blank,” add Diluent Solution (either distilled water or 10% DMSO in water, as described above).

5. Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. Three (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 kinase** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase to 0.8 ng/µl using **1x Kinase assay buffer**.

   Notes: the concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

   The kinase is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the diluted kinase.

8. Initiate the reaction by adding 20 µl of **diluted c-Met Kinase** to the wells designated “Positive Control” and "Test Inhibitor Control".

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Kinase Buffer	20 µl	-	-
c-Met Kinase	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

9. Incubate at 30˚C for 45 minutes.

10. During the incubation, thaw the Kinase-Glo Max reagent. At the end of the 45-minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

11. Immediately read in a luminometer or a microplate reader capable of reading luminescence. The “Blank” value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and 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: 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).

Example Results

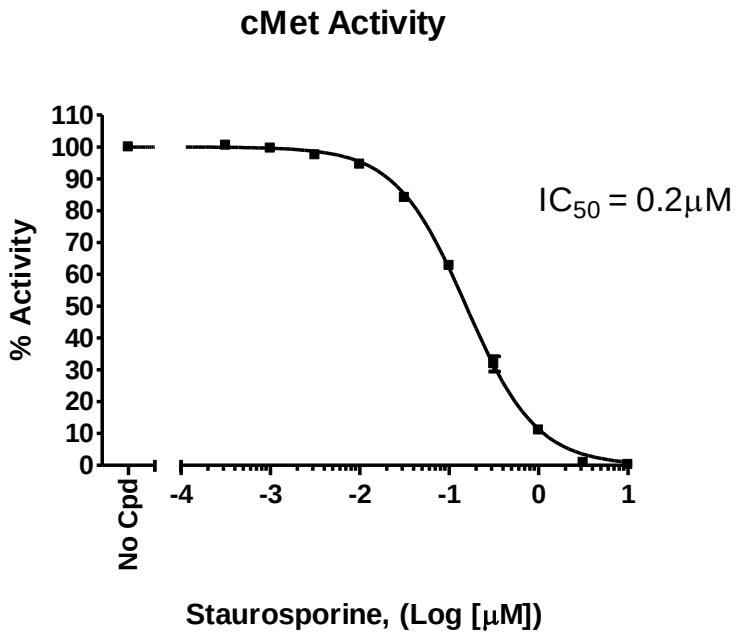

Figure 1: Inhibition of c-Met kinase Activity by Staurosporine. c-Met kinase activity was measured in the presence of increasing staurosporine concentrations using the c-Met Kinase Assay Kit (BPS Bioscience, #79559). The Blank value was subtracted from all other values. Results are expressed as percent of control (kinase activity in the absence of inhibitor, set at 100%).

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Recondo G. *et al*. Targeting *MET* Dysregulation in Cancer. *Cancer Discov*. 2020; 10(7): 922-934. Review.

Related Products

Products	*Catalog #*	*Size*
Human c-Met, GST-tag	40255	10 µg
Human c-MET (del 963-1009), GST-tag	100643	10 µg
Rat Met, GST-tag	40228	10 µg
c-Met (del 963-1009) Kinase Assay Kit	79930	96 reactions