EGFR (T790M, C797S) (del746-750) Kinase Assay Kit

#78595
96 reactions

Description

The EGFR (T790M, C797S) (del746-750) Kinase Assay Kit is designed to measure the kinase activity of mutant EGFR (T790M, C797S) (del746-750) 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 EGFR (T790M, C797S) (del746-750) kinase, kinase substrate, ATP and kinase assay buffer for 100 enzyme reactions.

Background

The EGFR (epidermal growth factor receptor), also known as ErbB-1 and HER1, is a cell surface tyrosine kinase receptor belonging to the epidermal growth factor receptor family. Overexpression and/or hyperactivation of EGFR kinase is associated with several human cancers such as lung, glioblastoma, and epithelial tumors of the neck and head. This increased activity of EGFR has led to the development of anticancer therapeutics targeting EGFR. However, d746-750, T790M, and C797S mutations in EGFR can cause resistance to known EGFR inhibitors, requiring the development of next generation mutant-selective inhibitors.

Application(s)

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

Supplied Materials

Catalog #	Name	Amount	Storage
100187	EGFR (T790M, C797S) (del746-750)*	2 µg	-80°C
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 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 Kinase Assay	Promega #V6071
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 TM 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 **PTK substrate 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 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase Assay Buffer 1** with 2400 µl water.
   *Note: Three (3ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare the **Master Mix** (25 µl/well): N wells x (6 µl of **5x Kinase Assay Buffer 1** + 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.

4. 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.

If the Test Inhibitor is water-soluble *(without DMSO):*

4.1 Prepare serial dilutions in the 1x Kinase Assay Buffer, 10-fold more concentrated than the desired final concentrations.

4.2 For the positive and negative controls, use **1x Kinase Assay Buffer** (Diluent Solution).

Or

If the Test inhibitor is soluble in DMSO (*with DMSO*):

4.1 Prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

4.2 Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer to keep the concentration of DMSO constant.

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

Note: The final concentration of DMSO should not exceed 1%.

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

6. To the wells designated as "Blank", add 20 µl of **1x Kinase Assay Buffer 1**.

7. Thaw **EGFR (T790M, C797S) (del746-750)** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase (20 µl/well) to 1 ng/µl using **1x Kinase Assay Buffer 1**.
   Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

   ⚠ *Note: This kinase is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.* ***Do not*** *re-use the thawed protein and do not re-use the diluted kinase.*

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

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 Assay Buffer	20 µl		
EGFR (T790M, C797S) (del746-750) (1ng/ µl)	-	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™. 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 at room temperature for 15 minutes.
11. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

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

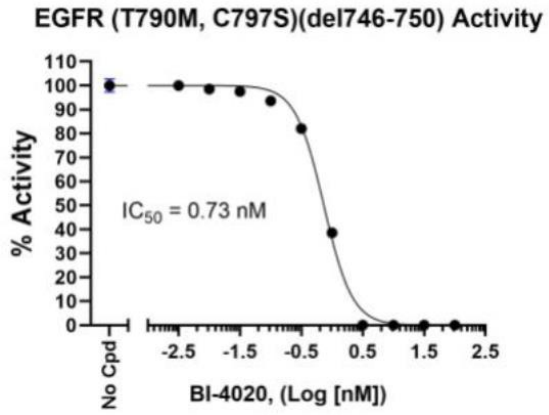

Figure 1: Inhibition of EGFR (T790M, C797S) (del746-750) kinase Activity by BI-4020.
The inhibition of EGFR (T790M, C797S) (del746-750) was measured using the EGFR (T790M, C797S) (del746-750) Kinase Assay Kit (BPS Bioscience #78595). % Activity = (blank - enzyme with inhibitor)/ (blank - enzyme without inhibitor)*100. Data shown is representative.

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
1. *Park, H. et al.* Angew Chem Int Ed Engl. 56(26):7634-7638 (2017).
2. Jia, Y., et al. *Nature* 534(7605) : 129–132 (2016).
3. Wang, S., *et al*. J. *Hematology & Oncology* 9:59 (2016).

Related Products

Products	*Catalog #*	*Size*
EGFR Kinase Assay Kit	40321	96 reactions
EGFR(T790M/L858R) Kinase Assay Kit	40322	96 reactions
Afatinib	27009	10 mg
Gefitinib	27032	1 g