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Data Sheet

EGFR (T790M/C797S/ L858R) Kinase Assay Kit

Catalog # 40326

DESCRIPTION: The epidermal growth factor receptor (EGFR; ErbB-1; HER1) is the cell-surface receptor for members of the epidermal growth factor family. Overexpression and/or hyperactivation of EGFR kinase is associated with several human cancers such as lung, glioblastoma, and epithelian tumors of the neck and head, leading to the development of anticancer therapeutics targeting EGFR. T790M, L858R and C797S mutations in EGFR cause resistance to known EGFR inhibitors. The *EGFR(T790M/C797S/L858R) Kinase Assay Kit* is designed to identify mutant-selective EGFR kinase inhibitors and to measure EGFR (T790M/C797S/L858R) kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *EGFR (T790M/C797S/L858R) Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant EGFR(T790M/C797S/L858R) enzyme, EGFR substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40351	EGFR (T790M/C797S/L858R)	2 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.

REFERENCES:
Wang, S., et al. J. Hematology & Oncology **9:**59 (2016)
Jia, Y., *et al. Nature* **534(7605):** 129–132 (2016).

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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	–
10% DMSO in 1x kinase assay buffer (inhibitor buffer)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
EGFR(T790M/C797S/ L858R) (1 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

1) 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 1x kinase assay buffer (inhibitor buffer). *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 a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

2) 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.

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

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4) Thaw **EGFR(T790M/C797S/L858R)** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **EGFR(T790M/C797S/L858R)** required for the assay and dilute enzyme to 1 ng/µl with 1x Kinase assay buffer. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **EGFR*(T790M/C797S/L858R)** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Initiate reaction by adding 20 µl of diluted **EGFR(T790M/C797S/L858R)** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 40 minutes.

6) Thaw Kinase-Glo Max reagent.

7) After the 45 minutes 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.

8) Measure luminescence using the microplate reader.

If preincubation of inhibitors with EGFR(T790M/C797S/L858R) is needed, mix the enzyme, water, 5X Kinase buffer and inhibitor first and preincubate the plate at room temperature. After preincubation, the reaction can be initiated by adding ATP and poly-Glu-Tyr substrate.

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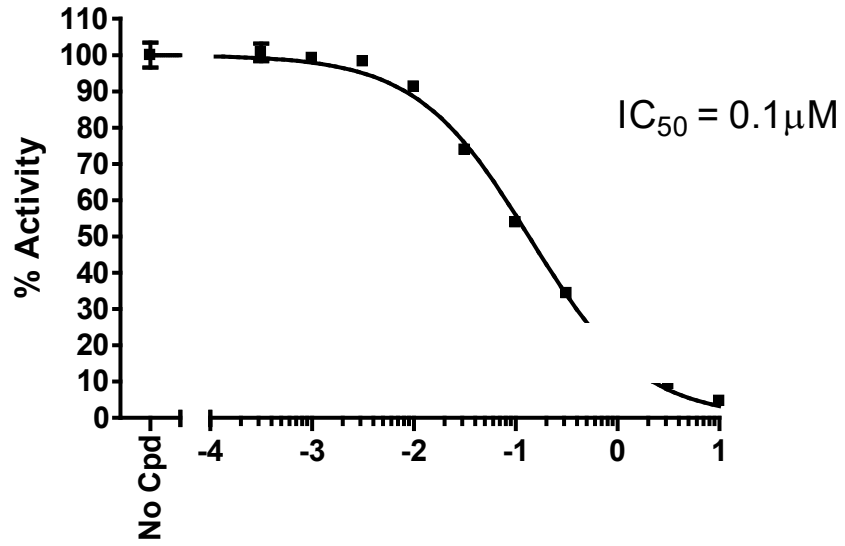
Example of Assay Results:

EGFR(L858R/T790M/C797S) Activity



Inhibition by AZD-9291. EGFR(T790M/C797S/L858R) was preincubated with AZD-9291 at room temperature. The reaction was initiated by adding ATP/poly-Glu-Tyr. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
EGFR	40187	10 µg
EGFR (L858R)	40189	10 µg
EGFR (T790M)	40188	10 µg
EGFR (T790M, L858R)	40350	10 µg
EGFR (T790M, C797S, L858R)	40351	10 µg
EGFR (mouse)	40195	10 µg
EGFR Kinase Assay Kit	40321	96 rxns.
EGFR(T790M/L858R) Kinase Assay Kit	40322	96 rxns.
Afatinib	27009	10 mg
Gefitinib	27032	1 g