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Data Sheet

EGFR(T790M) Kinase Assay Kit

Catalog # 40323

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. EGFR(T790M) mutation is found as a second-site mutation in ~50% of EGFR-mutated lung tumors. The mutant was shown to be resistant to most tyrosine kinase inhibitor medicines including Erlotinib and Gefitinib. The *EGFR(T790M) Kinase Assay Kit* is designed to measure EGFR (T790M) Kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *EGFR (T790M) Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant EGFR(T790M) enzyme, EGFR substrate, ATP and Kinase Buffer 1 for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40188	EGFR (T790M)	2 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase 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 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.

REFERENCE:

1. Nakamura, J.L. *Expert Opin. Ther. Targets* **11(4):**463-472 (2007)
2. Suda, K., *et al. J Thorac Oncol.* Jan;4(1):1-4 (2009).

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

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

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase Buffer 1** + 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 Buffer 1	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 (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
EGFR(T790M) (1 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (Inhibitor buffer).

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

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

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

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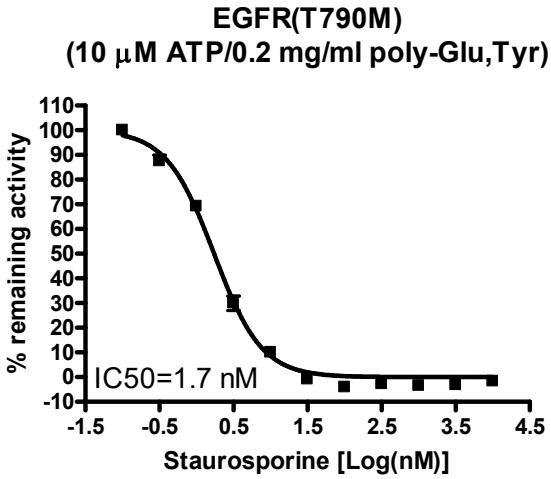
7) Initiate reaction by adding 20 µl of diluted **EGFR(T790M)** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 40 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.

10) Measure luminescence using the microplate reader.

Example of Assay Results:



Inhibition of wild-type EGFR(T790M) enzyme by Staurosporine, measured using the EGFR(T790M) kinase assay kit (Cat. #40323). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@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(T790M/L858R) Kinase Assay Kit	40322	384 rxns