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- Mindermengenzuschlag
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- Expressversand

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Data Sheet ***EGFR(T790M/L858R) Kinase Assay Kit*** **Catalog # 40322**

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 epithelial tumors of the neck and head, leading to the development of anticancer therapeutics targeting EGFR. EGFR(T790M/L858R) is an EGFR activating and drug resistance mutant that can be found in non-small-cell lung cancer cells. The *EGFR(T790M/L858R) Kinase Assay Kit* is designed to measure EGFR (T790M/L858R) Kinase activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The EGFR (T790M/L858R) Kinase Assay Kit comes in a convenient 96-well format, with enough purified recombinant EGFR(T790M/L858R) enzyme, EGFR substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40350	EGFR (T790M/L858R)	2 µg	-80°C	Avoid multiple freeze/thaw cycles!
	5x Kinase assay buffer	1.5 ml	-20°C	
	ATP (500 µM)	100 µl	-20°C	
40217	50x PTK substrate Poly(Glu:Tyr 4:1)	100 µl	Room Temp.	
	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:

Nakamura, J.L. *Expert Opin. Ther. Targets* **11(4)**:463-472 (2007)

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw 5x Kinase assay buffer, ATP and 50x PTK substrate.
(Optional: If desired, add DTT to 5x Kinase assay buffer to make a 10 mM concentration; e.g. add 10 μ l of 1 M DTT to 1 ml 5x Kinase assay buffer)
- 2) Prepare the master mixture (25 μ l per well): N wells x (6 μ l 5x Kinase assay buffer + 1 μ l ATP (500 μ M) + 1 μ l 50x PTK substrate + 17 μ l water). Add 25 μ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 μ l	6 μ l	6 μ l
ATP (500 μ M)	1 μ l	1 μ l	1 μ l
50x PTK substrate	1 μ l	1 μ l	1 μ l
Water	17 μ l	17 μ l	17 μ l
Test Inhibitor	-	5 μ l	-
Inhibitor Buffer (no inhibitor)	5 μ l	-	5 μ l
1x Kinase buffer	-	-	20 μ l
EGFR(T790M/858R) (1 ng/ μ l)	20 μ l	20 μ l	-
Total	50 μl	50 μl	50 μl

- 3) Add 5 μ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5 μ l of the same solution without inhibitor (Inhibitor buffer).
- 4) Prepare 3 ml of 1x Kinase assay buffer by mixing 600 μ l of 5x Kinase assay buffer with 2400 μ l water. 3 ml of 1x Kinase assay buffer is sufficient for 100 reactions.
- 5) To the wells designated as "Blank", add 20 μ l of 1x Kinase assay buffer.
- 6) Thaw EGFR(T790M/858R) enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of EGFR(T790M/858R) required for the assay and dilute enzyme to 1 ng/ μ l with 1x Kinase assay buffer. Store remaining undiluted enzyme in aliquots at -80°C. *Note: EGFR(T790M/858R) 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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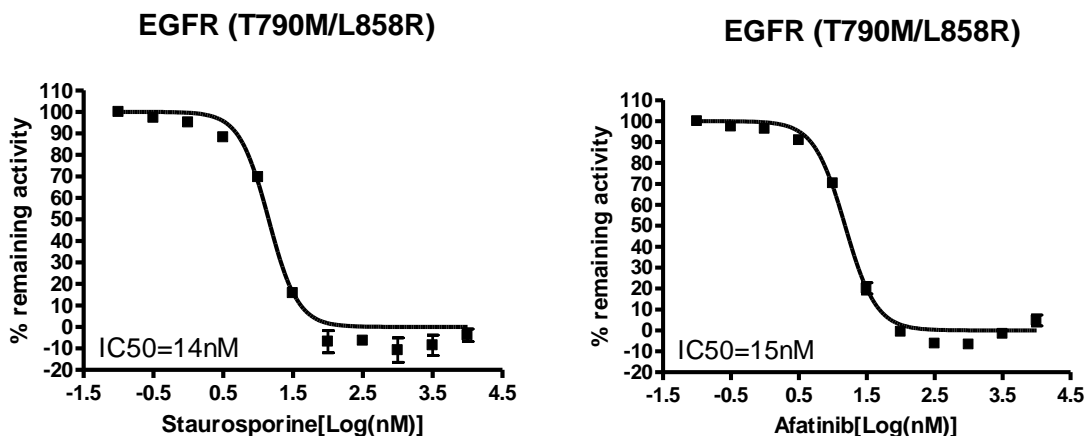
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- 7) Initiate reaction by adding 20 μ l of diluted EGFR(T790M/858R) enzyme to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30°C for 40 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 40 minute reaction, add 50 μ l 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/L858R) enzyme by Staurosporine (left) and Afatinib (right), measured using the EGFR(T790M/L858R) kinase assay kit (Cat. #40322). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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.

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