Met Positive Control
(Human, recombinant protein expressed in Sf9)
Cat# CY-E1080

Lot No.
For 100 Assays
(1 unit / µL x 100 µL)

Product Description:
Catalytic domain of human Met (HGF receptor), corresponding to 1076-1370a.a. containing a *N*-terminal GST tag and a C-terminal His tag, expressed in recombinant baculovirus infected sf9 cells. Purified by sequentially using GSH agarose and Ni-NTA agarose chromatography. The Met Positive control is designed to use for CycLex Met Kinase Assay/Inhibitor Screening Kit (Cat# CY-1080). The Met Positive Control should be added to the well at 1 unit/well. For instance, diluted positive control 1:10, use 10 µL for 1 assay. Unused Met Positive Control should be stored at -70°C.

Product Size: Recombinant Met: 100 units/100 µL

Formulation: The Met Positive Control is supplied frozen in a buffer containing 20mM Hepes-KOH (pH 7.5), 1 % BSA, 1mM EDTA, 2 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Source: Human Met containing N-terminal GST-tag and C-terminal His tag, expressed in sf9 cells.

Molecular Weight: Met Positive Control demonstrates a single 59 kDa bands by SDS-PAGE analysis.

Purity: Met Positive Control is greater than 75% pure as determined by SDS-PAGE analysis.

Substrates: Met phosphorylates poly[Glu, Tyr] 4:1 as a exogenous substrate.

Inhibitors: PHA-665752 and SU11274 are known as selective small molecule Met inhibitors [10, 11].

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1 nmol of phosphate into the Met (autophosphorylation) under oligomerized-activated condition per 60 minute at 30°C.

Assay Conditions: Assay activity of Met in a 50 µL reaction containing 20 mM Hepes KOH (pH 7.5), 4 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM DTT, 50 µM [gamma ^{32}P] ATP (1 µCi), and 4 µg of CycLex-"Tyrosine kinase-binding module". Start the reaction by adding 10µL of the enzyme, diluted 10-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT, 0.03 % Brij35. Incubate for 60 minutes at 30°C. Terminate the reaction by adding 600 µL of cold 10 % TCA solution containing 0.2 % Sodium pyrophosphate and stand on ice for 15 min. Filtrate acid insoluble material through GFC filters (Whattman Inc.), wash 4 times with 1 % TCA and rinse filters with ethanol. Dry filters and count in a liquid scintillation counter.

Storage and Stability: Stable for 12 months at **-70°C** from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot enzyme to avoid repeated freezing and thawing.

Related Products:
* CycLex Met Kinase Assay/Inhibitor Screening Kit: Cat# CY-1080

References:
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