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

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

Product Description:
Catalytic domain of human Met (HGF receptor), corresponding to 1076-1370 a.a. containing an *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: 100 units/100 µL

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

References:
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