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Certificate of Analysis

Met (D1246H), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-915, 14-915-K, 14-915M
Parent Lot # D7SN029N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, human, Met amino acids 974–end, containing the mutation D1246H, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose.
Met D1246H is a somatic mutation that has been identified in patients with papillary renal carcinomas (HPRC). *In vitro* studies have demonstrated enhanced kinase activity from this mutant. (Schmidt *et al*, (1997), Nature Genetics, **16**: 68-73 and Jeffers *et al* (1997), PNAS, **94**: 11445-11450).
Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 50.1kDa.

Specific Activity (Parent lot # D7SN029N): 1450U/mg, where one unit of Met (D1246H), active activity is defined as 1nmol phosphate incorporated into 250µM (KKKGQEEEYVFIE) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.069mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

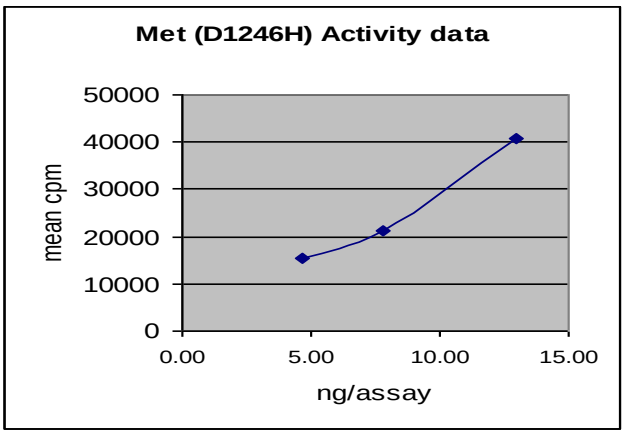
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

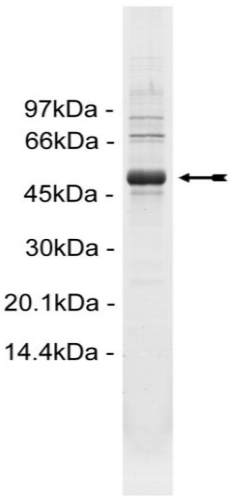
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 4.7–12.9ng of this lot of enzyme phosphorylated 250µM (KKKGQEEEYVFIE) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Met with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Met (D1246H), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Na_3VO_4:** Use at a final assay concentration of 1mM. Prepare a 100mM stock and add 0.25µl of stock per assay point.
3. **Na-β-glycerophosphate:** Use at a final assay concentration of 5mM. Prepare a 1M stock and add 0.125µl of stock per assay point.
4. **(KKKGQEEEYVFIE):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
5. **Met (D1246H), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4.7–12.9ng per assay point.
6. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKKGQEEEYVFIE)**.
3. Add **2.5µl (4.7–12.9ng) Met (D1246H), active**.
4. Add 4.625µl of dH_2O.
5. Add 0.25µl Na_3VO_4.
6. Add 0.125µl Na-β-glycerophosphate.
7. Add 10µl of diluted [γ-^{33}P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Met (D1246H) Sequence Information

Protein Human Met

Tags *N*-terminal 6His

Native sequence K8 of the recombinant protein is equivalent to K974 of human Met

Accession number GenBank J02958. The recombinant protein contains the amino acid substitutions A1209G and V1290L with reference to GenBank J02958. Both substitutions are reported in GenBank BU595386, BQ315895 and BQ316491.

Recombinant Met (D1246H) amino acid sequence:

```
   1 MHHHHHHKKR KQIKDLGSEL VRYDARVHTP HLDRLVSARS VSPTTEMVSN ESVDYRATFP
  61 EDQFPNSSQN GSCRQVQYPL TDMSPILTSG DSDISSPLLQ NTVHIDLSAL NPELVQAVQH
 121 VVIGPSSLIV HFNEVIGRGH FGCVYHGTLL DNDGKKIHCA VKSLNRITDI GEVSQFLTEG
 181 IIMKDFSHPN VLSLLGICLR SEGSPLVVLP YMKHGDLRNF IRNETHNPTV KDLIGFGLQV
 241 AKGMKYLASK KFVHRDLAAR NCMLDEKFTV KVADFGLARH MYDKEYYSVH NKTGAKLPVK
 301 WMALESLQTQ KFTTKSDVWS FGVLLWELMT RGAPPYPDVN TFDITVYLLQ GRRLLQPEYC
 361 PDPLYEVMLK CWHPKAEMRP SFSELVSRIS AIFSTFIGEH YVHVNATYVN VKCVAPYPSL
 421 LSSEDNADDE VDTRPASFWE TS
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Recombinant Met (D1246H) nucleotide sequence:

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   1 atgcatcacc atcaccatca taaaaagcga aagcaaatta aagatctggg cagtgaatta
  61 gttcgctacg atgcaagagt acacactcct catttggata ggcttgtaag tgcccgaagt
 121 gtaagcccaa ctacagaaat ggtttcaaat gaatctgtag actaccgagc tacttttcca
 181 gaagatcagt ttcctaattc atctcagaac ggttcatgcc gacaagtgca gtatcctctg
 241 acagacatgt cccccatcct aactagtggg gactctgata tatccagtcc attactgcaa
 301 aatactgtcc acattgacct cagtgctcta aatccagagc tggtccaggc agtgcagcat
 361 gtagtgattg ggcccagtag cctgattgtg catttcaatg aagtcatagg aagagggcat
 421 tttggttgtg tatatcatgg gactttgttg gacaatgatg gcaagaaaat tcactgtgct
 481 gtgaaatcct tgaacagaat cactgacata ggagaagttt cccaatttct gaccgaggga
 541 atcatcatga aagattttag tcatcccaat gtcctctcgc tcctgggaat ctgcctgcga
 601 agtgaagggt ctccgctggt ggtcctacca tacatgaaac atggagatct tcgaaatttc
 661 attcgaaatg agactcataa tccaactgta aaagatctta ttggctttgg tcttcaagta
 721 gccaaaggca tgaaatatct tgcaagcaaa aagtttgtcc acagagactt ggctgcaaga
 781 aactgtatgc tggatgaaaa attcacagtc aaggttgctg attttggtct tgccagacac
 841 atgtatgata aagaatacta tagtgtacac aacaaaacag gtgcaaagct gccagtgaag
 901 tggatggctt tggaaagtct gcaaactcaa aagtttacca ccaagtcaga tgtgtggtcc
 961 tttggcgtgc tcctctggga gctgatgaca agaggagccc caccttatcc tgacgtaaac
1021 acctttgata taactgttta cttgttgcaa gggagaagac tcctacaacc cgaatactgc
1081 ccagacccct tatatgaagt aatgctaaaa tgctggcacc ctaaagccga aatgcgccca
1141 tccttttctg aactggtgtc ccggatatca gcgatcttct ctactttcat tggggagcac
1201 tatgtccatg tgaacgctac ttatgtgaac gtaaaatgtg tcgctccgta tccttctctg
1261 ttgtcatcag aagataacgc tgatgatgag gtggacacac gaccagcctc cttctgggag
1321 acatcatag
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Reviewed and approved by site quality representative.