

Certificate of Analysis

Met (D1246N), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-818, 14-818-K, 14-818M

Parent Lot # D8AN014N

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 D1246N, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose.

Met D1246N is a germline mutation that has been identified in patients with hereditary 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 81% by SDS-PAGE and Coomassie blue staining. MW = 50.1kDa.

Formulation: 1.265mg/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.

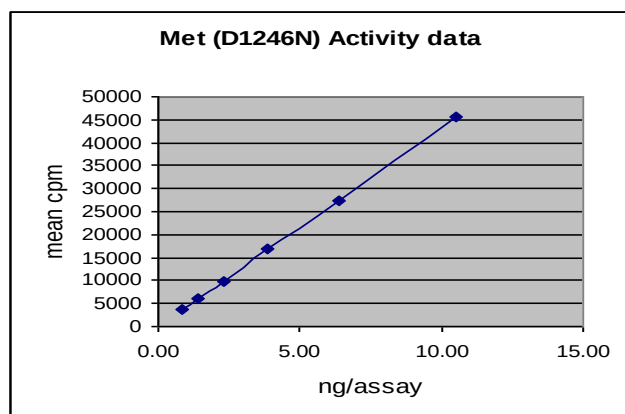
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

Specific Activity (Parent lot# D8AN014N): 2160U/mg, where one unit of Met (D1246N), active activity is defined as 1nmol phosphate incorporated into 250µM (KKKGQEEEEYVFIE) per minute at 30°C with a final ATP concentration of 100µM.

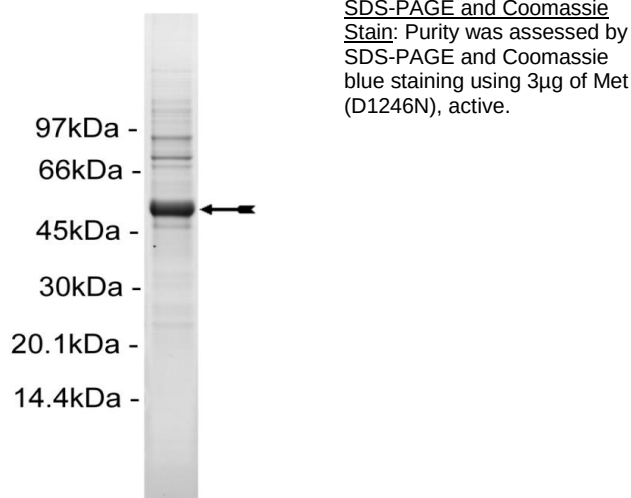
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.8–10.5ng of this lot of enzyme phosphorylated 250µM (KKKGQEEEEYVFIE) 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.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Na₃VO₄:** 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. **(KKKGQEEYVFIE):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
5. **Met (D1246N), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.8–10.5ng per assay point.
6. **[γ-³³P]ATP:** 2.5 x MgAc/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]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 **(KKKGQEEYVFIE)**.
3. Add **2.5µl (0.8–10.5ng) Met (D1246N), active**.
4. Add 4.625µl of dH₂O.
5. Add 0.25µl Na₃VO₄.
6. Add 0.125µl Na-β-glycerophosphate.
7. Add 10µl of diluted [γ-³³P]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 (D1246N) Sequence Information

<u>Protein</u>	Human Met
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	K8 of the recombinant protein is equivalent to K974 of human Met
<u>Accession number</u>	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 (D1246N) amino acid sequence:

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1  MHHHHHHKKR  KQIKDLGSEL  VRYDARVHTP  HLDRLVSARS  VSPTTEMVSN  ESVDYRATFP
61  EDQFPNSSQN  GSCRQVQYPL  TDMSPILTSG  DSDISSPLLQ  NTVHIDLSAL  NPELVQAVQH
121 VVIGPSSLIV  HFNEVIGRGH  FGCVYHGTL  DNDGKKIHCA  VKSLNRITDI  GEVSQFLTEG
181 IIMKDFSHPN  VLSLLGICLR  SEGSPVLVLP  YMKHGDLRNF  IRNETHNPTV  KDLIGFGLQV
241 AKGMKYLASK  KFVHRDLAAR  NCMLDEKFTV  KVADFGLARN  MYDKEYYSVH  NKTGAKLPVK
301 WMALES LQTQ  KFTTKSDVWS  FGVLLWELMT  RGAPPYPDVN  TFDITVYLLQ  GRRLLQPEYC
361 PDPLYEVMLK  CWHPKAEMRP  SFSELVSRI  AIFSTFIGEH  YVHV NATYVN  VKCVAPYPSL
421 LSEDNADDE  VDTRPAS FWE  TS

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Recombinant Met (D1246N) nucleotide sequence:

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1  atgcatcacc  atcaccatca  taaaaagcga  aagcaaatta  aagatctggg  cagtgaatta
61  gttcgctacg  atgcaagagt  acacactcct  catttgata  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  cagtgtctta  aatccagagc  tgggtccaggc  agtgcagcat
361 gtagtgattg  ggcccagtag  cctgattgtg  catttcaatg  aagtcatagg  aagagggcat
421 tttggttggt  tatacatggt  gactttgttg  gacaatgatg  gcaagaaaat  tcaactgtgt
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541 atcatcatga  aagattttag  tcatcccaat  gtcctctcgc  tcctgggaat  ctgcctgcga
601 agtgaagggt  ctccgctggt  ggtcctacca  tacatgaaac  atggagatct  tcgaaatttc
661 attcgaatg  agatcataa  tccaactgta  aaagatctta  ttggctttgg  tcttcaagta
721 gccaaaggga  tgaatatct  tgcaagcaaa  aagtttgctc  acagagactt  ggctgcaaga
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841 atgtatgata  aagaatacta  tagtgtacac  aacaaaacag  gtgcaaaagt  gtcagtgaag
901 tggatggctt  tggaaagtct  gcaaactcaa  aagtttacca  ccaagtcaga  tgtgtggtcc
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1021 acctttgata  taactgttta  cttgttgcaa  gggagaagac  tcctacaacc  cgaatactgc
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1201 tatgtccatg  tgaacgtac  ttatgtgaac  gtaaaatgtg  tcgtccgta  tccttctctg
1261 ttgtcatcag  aagataacgc  tgatgatgag  gtggacacac  gaccagcctc  cttctgggag
1321 acatcatag

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Reviewed and approved by site quality representative.

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