

Certificate of Analysis

Met (M1268T), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-817, 14-817-K, 14-817M

Parent Lot # D7SN049N

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 M1268T, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose.

Met M1268T 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 71% by SDS-PAGE and Coomassie blue staining. MW = 50.1kDa.

Formulation: 0.669mg/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.

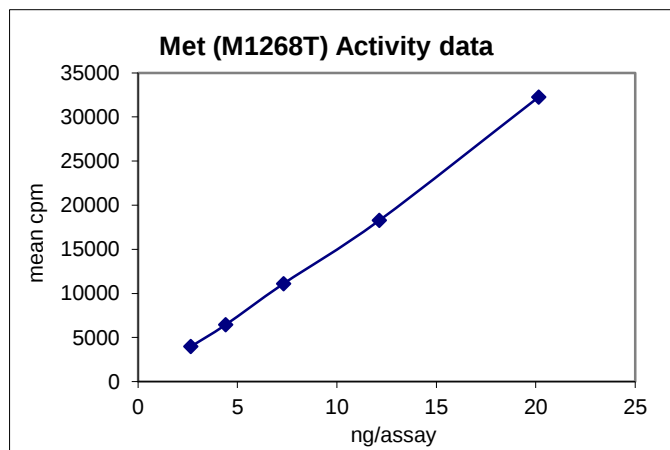
Specific Activity (Parent lot# D7SN049N): 741U/mg, where one unit of Met (M1268T), active activity is defined as 1nmol phosphate incorporated into 250μM (KKKGQEEEEYVFIE) per minute at 30°C with a final ATP concentration of 100μM.

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.

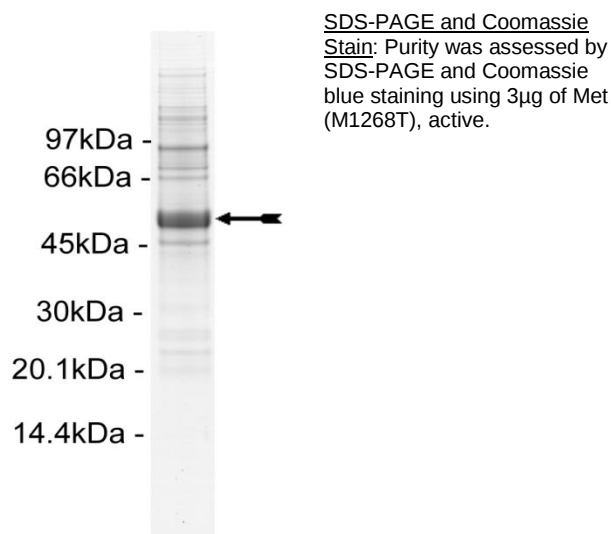
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3–20ng 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.



Certificate of Analysis

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. **(KKKGQEEEEYVFIE):** Use at a final assay concentration of 250 µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
5. **Met (M1268T), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 3–20ng 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 **(KKKGQEEEEYVFIE)**.
3. Add **2.5µl (3–20ng) Met (M1268T), 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.

Certificate of Analysis

Met (M1268T) 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 (M1268T) amino acid sequence:

```

1 MHHHHHHKKR KQIKDLGSEL VRYDARVHTP HLDRLVSARS VSPTTEMVSN ESVDYRATFP
61 EDQFPNSSQN GSCRQVQYPL TDMSPILTS GSDISSPLLQ NTVHIDLSAL NPELVQAVQH
121 VVIGPSSLIV HFNEVIGRGH FGCVYHGTL DNDGKKIHCA VKSLNRITDI GEVSQFLTEG
181 IIMKDFSHPN VLSLLGICLR SEGSPVLVLP YMKHGD L RNF IRNETHNPTV KDLIGFGLQV
241 AKGMKYLASK K FVHRDLAAR NCMLDEKFTV KVADFLGLARD MYDKEYYSVH NKTGAKLPVK
301 WTALES LQTQ KFTTKSDVWS FGVLLWELMT RGAPPYPDVN TFDITVYLLQ GRRL LQPEYC
361 PDPLYEVMLK CWHPKAEMRP SFSELVSRIS AIFSTFIGEH YVHV NATYVN VKCVAPYPSL
421 LSSEDNADDE V DTRPAS FWE TS

```

Recombinant Met (M1268T) nucleotide sequence:

```

1 atgcatcacc atcaccatca taaaaagcga aagcaaatta aagatctggg cagtgaatta
61 gttcgctacg atgcaagagt acacactcct catttgata ggcttgtaag tgcccgaagt
121 gtaagcccaa ctacagaaat ggtttcaaat gaatctgtag actaccgagc tacttttcca
181 gaagatcagt ttctaattc atctcagaac ggttcatgcc gacaagtgca gtatcctctg
241 acagacatgt ccccatcct aactagtggg gactctgata tatccagtc attactgcaa
301 aatactgtcc acattgacct cagtgtctta aatccagagc tgggtccaggc agtgcagcat
361 gtagtgattg ggcccagtag cctgattgtg catttcaatg aagtcatagg aagagggcat
421 tttgggtgtg tatatcatgg gactttgttg gacaatgatg gcaagaaaat tcaactgtgt
481 gtgaaatcct tgaacagaat cactgacata ggagaagttt cccaatttct gaccgaggga
541 atcatcatga aagattttag tcatcccaat gtcctctcgc tcctgggaat ctgcctgcga
601 agtgaagggt ctccgctggg ggtcctacca tacatgaaac atggagatct tcgaaatttc
661 attcgaaatg agactcataa tccaactgta aaagatctta ttggctttgg tcttcaagta
721 gccaaaggca tgaaatatct tgcaagcaaa aagtttgtcc acagagactt ggctgcaaga
781 aactgtatgc tggatgaaaa attcacagtc aaggttgctg attttggctt tgccagagac
841 atgtatgata agaataacta tagtgtacac aacaaaacag gtgcaaagct gccagtgaag
901 tggacggctt tggaaagtct gcaaactcaa aagtttacca ccaagtcaga tgtgtggtcc
961 tttggcgtgc tcctctggga gctgatgaca agaggagccc caccttatcc tgacgtaaac
1021 acctttgata taactgttta cttgttgcaa gggagaagac tcctacaacc cgaatactgc
1081 ccagaccctt tatatgaagt aatgctaaaa tgctggcacc cttaaagccga aatgcgcca
1141 tccttttctg aactggtgtc ccgatatca gcgatcttct ctactttcat tggggagcac
1201 tatgtccatg tgaacgctac ttatgtgaac gtaaaatgtg tcgctccgta tccttctctg
1261 ttgtcatcag aagataacgc tgatgatgag gtggacacac gaccagcctc cttctgggag
1321 acatcatag

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.