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## Certificate of Analysis

### Met, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-526M

Lot # D8EN017U

**Product Description:** N-terminal 6His-tagged recombinant human Met residues 974–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA-agarose. Purity 79.7% by SDS-PAGE and Coomassie blue staining. MW = 50kDa.

**Specific Activity (lot# D8EN017U)** 351U/mg, where one unit of Met activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 250µg of enzyme in 84.5µl of 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

**Storage and Stability:** Store at -70°C. For maximum recovery of product, centrifuge original vial prior to removing the cap.

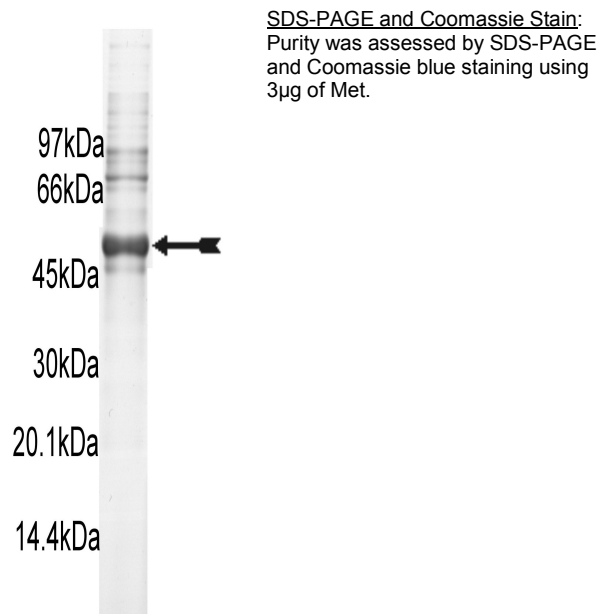
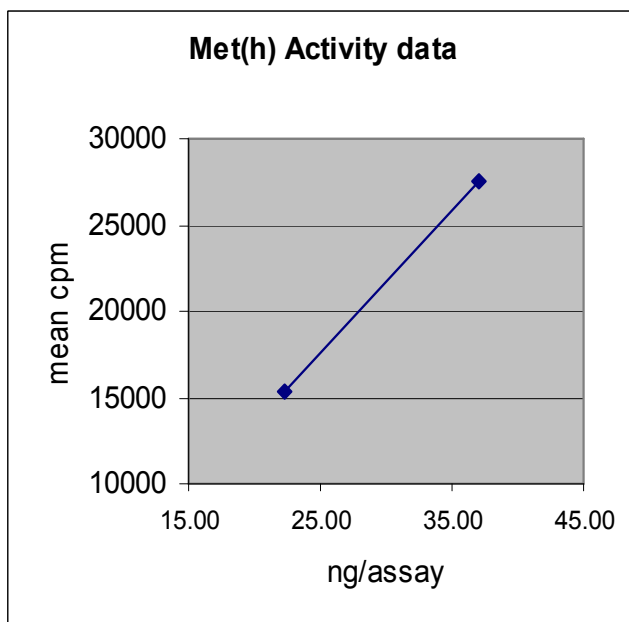
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

### Quality Control Testing

**Kinase Assay:** 22–36ng of this lot of enzyme phosphorylated 250µM in the assay described on page two. Assay background was subtracted from the actual counts to yield the results.

**MS Tryptic Fingerprint:** Confirmed identity Met with 28% amino acid coverage of the translated sequence listed on page three.



## Kinase Assay Protocol

### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 25 $\mu$ M. Prepare a 2.5mM stock and add 2.5 $\mu$ l of stock per assay point.
3. **Met, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 22–36ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **(KKKSPGEYVNIEFG)**.
3. Add **2.5 $\mu$ l (22–36ng) Met, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 $\mu$ l of 30% phosphoric acid.

## Met Sequence Information

<b>Protein</b>	Human Met
<b>Tags</b>	N-terminal 6His
<b>Native sequence</b>	K8 of the recombinant protein is equivalent to K974 of human Met
<b>Accession number</b>	GenBank J02958. This construct contains the conflicts A1209G and V1290L with respect to GenBank J02958. Both conflicts are reported in GenBank BU595386, BQ315895 and BQ316491.

### Recombinant Met amino acid sequence:

```
1  MHHHHHHKKR  KQIKDLGSEL  VRYDARVHTP  HDRLVLSARS  VSPTTEMVSN  ESVDYRATFP
61  EDQFPNSSQN  GSCRQVQYPL  TDMSPILTSG  DSDISSPLLQ  NTVHIDLSAL  NPELVQAVQH
121 VVIGPSSLIV  HFNEVIGRGH  FGCYVHGTL  DNDGKKIHCA  VKSLNRITDI  GEVSQFLTEG
181 IIMKDFSHPN  VLSLLGICLR  SEGSPLVVLP  YMKHGDLRNF  IRNETHNPTV  KDLIGFGLQV
241 AKGMKYLASK  KVVHRDLAAR  NCMLDEKFTV  KVADFGLARD  MYDKEYYSVH  NKTGAKLPVK
301 WMALESLOTQ  KFTTKSDVWS  FGVLLWELMT  RGAPPYPDVN  TFDITVYLLQ  GRRLQPEYEC
361 PDPLYEVMLK  CWHPKAEMRP  SFSELVSRIS  AIFSTFIGEH  YVHVNATYVN  VKCVAPYPSL
421 LSSEDNADDE  VDTRPASFWE  TS
```

### Recombinant Met 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  ttcctaattc  atctcagaac  ggttcatgcc  gacaagtgca  gtatcctctg
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361 gtagtgattg  ggcccagtag  cctgattgtg  catttcaatg  aagtcatagg  aagagggcat
421 tttggttg  tataatcatgg  gactttgtg  gacaatgatg  gcaagaaaat  tcaactgtgct
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1261 ttgtcatcag  aagataacgc  tgatgatgag  gtggacacac  gaccagcctc  cttctgggag
1321 acatcatag
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