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Certificate of Analysis

Met, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-526

Lot # 1616806

From bulk lot # D8PN031U

Product Description: N-terminal 6His-tagged recombinant human Met residues 974–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 58.9% by SDS-PAGE and Coomassie blue staining. MW = 50kDa.

Specific Activity (lot# 1616806): 509U/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: 10µg of enzyme in 2.7µl 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.

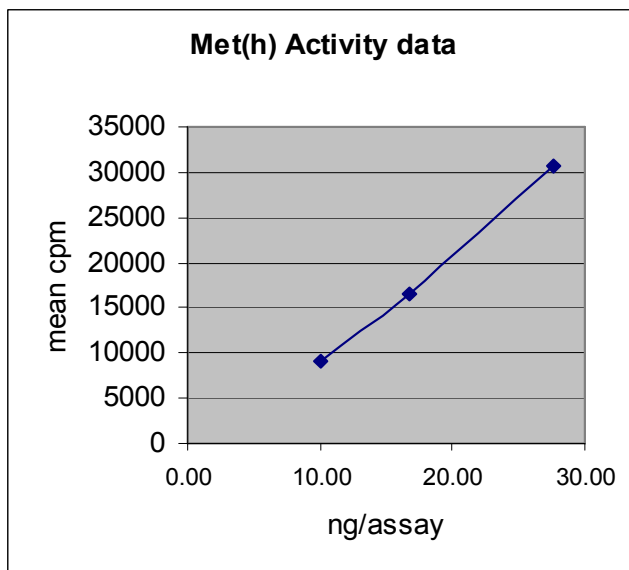
Storage and Stability: Store at -70°C. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

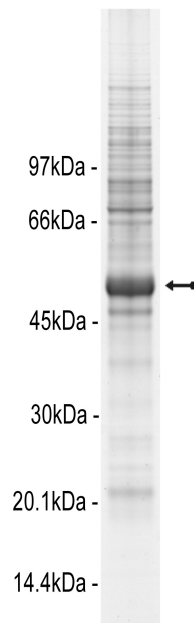
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 10.1–27.7ng 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 35% amino acid coverage of 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.

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 μ M. Prepare a 2.5mM stock and add 2.5 μ 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 10.1–27.7ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKKSPGEYVNIEFG)**.
3. Add **2.5 μ l (10.1–27.7ng) Met, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ 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 μ l of 30% phosphoric acid.

Met 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. 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:

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1 MHHHHHHKKR KQIKDLGSEL VRYDARVHTP HLDRLVSARS VSPTTEMVSN ESVDYRATFP
61 EDQFPNSSQN GSCRQVQYPL TDMSPIILTSG DSDISSPLLQ NTVHIDL SAL NPELVQAVQH
121 VVIGPSSLIV HFNEVIGRGH FGCYVHG TLL DNDGKKIHCA VKSLNRITDI GEVSQFLTEG
181 IIMKDFSHPN VLSLLGICLR SEGSPLVVLP YMKHGD LRFN IRNETHNPTV KDLIGFGLQV
241 AKGMKYLASK KFHVRDLAAR NCMLDEKFTV KVADFGLARD MYDKEYYSVH NKTGAKLPVK
301 WMALES LQTQ KFTTKSDVWS FGVLLWELMT RGAPPYPDVN TFDITVYLLQ GRRL LQPEYC
361 PDPLYEVMLK CWHPKAEMRP SFSELVSRIS AIFSTFIGEH YVHV NATYVN VKCVAPYPSL
421 LSSEDNADDE VDTRPAS FWE TS
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Recombinant Met 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 tatccagtc attactgcaa
301 aatactgtcc acattgacct cagtgtctta aatccagagc tggtcaggc agtgcagcat
361 gtagtgattg ggcccagtag cctgattgtg catttcaatg aagtcatagg aagagggcat
421 tttggttggt tataatcatgg gactttgttg gacaatgatg gcaagaaaat tcaactgtgt
481 gtgaaatcct tgaacagaat cactgacata ggagaagttt cccaatttct gaccgagggg
541 atcatcatga aagattttag tcatcccaat gtcctctcgc tcttggaat ctgctgcga
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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.

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