

# Certificate of Analysis

## MET M1250T

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0982-0000-1

Lot: 002

**Description:** Human MET C-terminal fragment, amino acids K<sub>956</sub>-S<sub>1390</sub> (as in NCBI/Protein entry NP\_000236.2), M1250T mutant, N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** MET M1250T Lot 002 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 78,757 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

**Activation:** This kinase was not activated by special procedures

**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

**Storage temperature:** -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.504 µg/µl

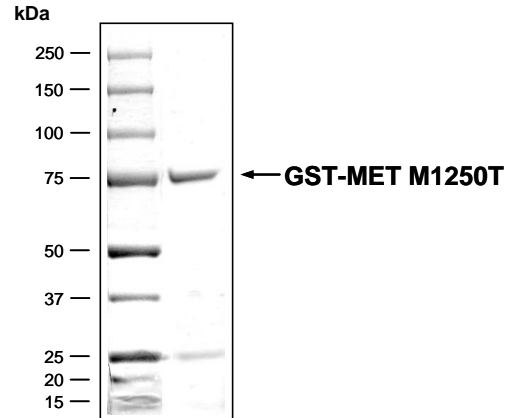
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

Specific kinase activity (P<sub>i</sub> transfer): 97 pmol/µg × min

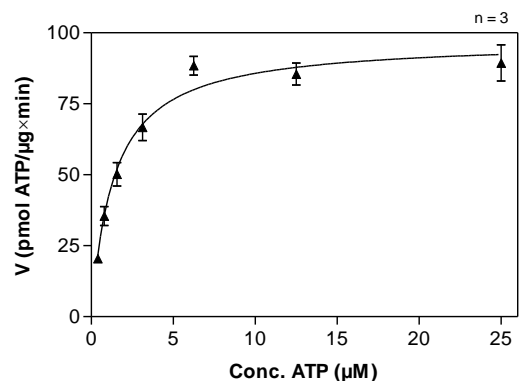
ATP-K<sub>M</sub>: 1.4 µM

**MET M1250T Lot 002:**  
**Coomassie stain**



2.0 µg GST-MET M1250T

**MET M1250T Lot 002:**  
**Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP**



**Determination of K<sub>M</sub> value & Specific activity:**

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: TRK-C derived peptide 20 µg/ml
  - Kinase: 1.0 µg/ml
- Filter binding assay
  - MSPH membrane (Millipore)

**Additional assay technology:** MET M1250T Lot 002

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from



ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

# MET M1250T

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MET M1250T Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYYY	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHH</b> HG	RRRASVAAGI	240
241	<b>LVPRG</b> SPGLD	GICSIEEFKK	<b>RKQIKDLGSE</b>	<b>LVRVDARVHT</b>	<b>PHLDRLVSAR</b>	<b>SVSPTTEMVS</b>	300
301	<b>NESVDYRATF</b>	<b>PEDQFPNSSQ</b>	<b>NGSCRQVQYP</b>	<b>LTDMSPI LTS</b>	<b>GSDDISSPLL</b>	<b>QNTVHIDL SA</b>	360
361	<b>LNPELVQAVQ</b>	<b>HVVI GPSSLI</b>	<b>VHFNEVIGRG</b>	<b>HFGCVYHGTL</b>	<b>LDNDGKKIHC</b>	<b>AVKSLNRITD</b>	420
421	<b>IGEVSQFLTE</b>	<b>GIIMKDFSH P</b>	<b>NVLSLLGICL</b>	<b>RSEGSPLVVL</b>	<b>PYMKHGDLRN</b>	<b>FIRNETHNPT</b>	480
481	<b>VKDLIGFGLQ</b>	<b>VAKGMKYLAS</b>	<b>KKFVHRDLAA</b>	<b>RNCMLDEKFT</b>	<b>VKVADFGLAR</b>	<b>DMYDKEYYSV</b>	540
541	<b>HNKTGAKLPV</b>	<b>KWTALESLOT</b>	<b>QKFTTKSDVW</b>	<b>SFGVLLWELM</b>	<b>TRGAPPYPDV</b>	<b>NTFDITVYLL</b>	600
600	<b>QGRLLQPEY</b>	<b>CPDPLYEVML</b>	<b>KCWHPKAEMR</b>	<b>PSFSELVSRI</b>	<b>SAIFSTFIGE</b>	<b>HYVHV NATYV</b>	660
661	<b>NVKCVAPYPS</b>	<b>LLSSEDNADD</b>	<b>EVDTRPASFW</b>	<b>ETS</b>			720

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: MET fragment **boxed**: M1250T

MET wt <sup>1</sup> Amino Acid Sequence							
1	MKPAVAVLAPG	ILVLLFTLVQ	RSNGECKEAL	AKSEMNVNMK	YQLPNFTAET	PIQNVILHEH	60
61	HIFLGATNYI	YVLNEEDLQK	VAEYKTGPVL	EHPDCFPQD	CSSKANLSGG	VWKDNI NMAL	120
121	VVDTYDDQL	ISCGSVNRGT	CQRHVFPNH	TADIQSEVHC	IFSPQIEEPS	QCPDCVVSAL	180
181	GAKVLSSVKD	RFINFFVGNT	INSSYFPDHP	LHSISVRLK	ETKDGFMFLT	DQSYIDV LPE	240
241	FRDSYPIKYV	HAFESNNFIY	FLTQVRETLD	AQTFHTRIIR	FCSINSLGHS	YMEMPL ECIL	300
301	TEKRKRSTK	KEVFNILQAA	YVSKPGAQLA	RQIGASLNDD	ILFGVFAQSK	PDSAEPMDRS	360
361	AMCAFPKIYV	NDFFNKIVNK	NNVRCLQHFY	GNPHEHC FNR	TLLRNSSGCE	ARRDEYRTEF	420
421	TTALQRVDLF	MGQFSEVLLT	SISTFIKGD L	TIANLGTSEG	RFMQVVVSR S	GPSTPHVNFL	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGKKI	TKIPLNGLGC	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQOI	CLPAIYKVPF	NSAPLEGGTR	LTICGWDFGF	RRNNKFDLKK	600
600	TRVLLGNESC	TLTLSESTMN	TLKCTVGPAM	NKHFNM SIII	SNGHGTTQYS	TFSYVDPVIT	660
661	SISPKYGPMA	GGTLLTLTGN	YLN SGN SRHI	SIGGKTCTLK	SVSNSILECY	TPAQTI STEF	720
721	AVKLIKIDLAN	RETSIFS YRE	DPIVYEIHPT	KSFISGGSTI	TGVGKNLNSV	SVPRMVINVH	780
781	EAGRNFTVAC	QHRSNSEIIC	CTTPSLQQLN	LQLPLKTKAF	FMLDGILSKY	FDLIYVHN PV	840
841	FKPFEKPVMI	SMGNE NVLEI	KGNDIDPEAV	KGEVLKVG NK	SCENIHLHSE	AVLCTV PNDL	900
901	LKLNSELNIE	WKQAISSTVL	GKVIVQPDQN	FTGLIAGVVS	ISTALLLLLG	FFLWL <b>KKRKQ</b>	960
961	<b>IKDLGSELVR</b>	<b>YDARVHTPHL</b>	<b>DRLVSARSVS</b>	<b>PTTEMVSNES</b>	<b>VDYRATFPED</b>	<b>QFPNSSQNGS</b>	1020
1021	<b>CRQVQYPLTD</b>	<b>MSPILTS GDS</b>	<b>DISSPLLQNT</b>	<b>VHIDLSALNP</b>	<b>ELVQAVQHVV</b>	<b>IGPSSLI VHF</b>	1080
1081	<b>NEVIGRGHFG</b>	<b>CVYHGTL LDN</b>	<b>DGKKIHCAVK</b>	<b>SLNRITDIGE</b>	<b>VSQFLTEGII</b>	<b>MKDFSHPNVL</b>	1140
1141	<b>SLLGICLRSE</b>	<b>GSPLVLPYM</b>	<b>KHGDLRNFIR</b>	<b>NETHNPTVKD</b>	<b>LIGFGLQVAK</b>	<b>GMKYLASKKF</b>	1200
1201	<b>VHRDLAARNC</b>	<b>MLDEKFTVKV</b>	<b>ADFGLARDMY</b>	<b>DKEYYSVH NK</b>	<b>TGAKLPVKWM</b>	<b>ALESLOTQKF</b>	1260
1261	<b>TTKSDVWSFG</b>	<b>VLLWELMTRG</b>	<b>APPYPDVNTF</b>	<b>DITVYLLQGR</b>	<b>RLLQPEYCPD</b>	<b>PLYEVMLK CW</b>	1320
1321	<b>HPKAEMRPSF</b>	<b>SELVSRISAI</b>	<b>FSTFIGEHYV</b>	<b>HVNATYVNVK</b>	<b>CVAPYPSLLS</b>	<b>SEDNADDEVD</b>	1380
1381	<b>TRPASFWETS</b>						1440

**blue**: MET sequence expressed in fusion protein **Red**: variant in fusion protein

<sup>1</sup>NCBI/Protein accession number NP\_000236.2

**Please notice:**

Variant amino acid numbering beginning with Ser755 when referring to GenBank accession J02958 (additional 18 aa exon between S755/G756, frequently found in the literature)