

# Certificate of Analysis

## MET F1200I

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0978-0000-1

Lot: 001

**Description:** Human MET C-terminal fragment, amino acids K<sub>956</sub>-S<sub>1390</sub> (as in NCBI/Protein entry NP\_000236.2), F1200I mutant, N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** MET F1200I Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 78,753 Da

**Expression:** Baculovirus infected Sf9 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:** 0.307 µg/µl

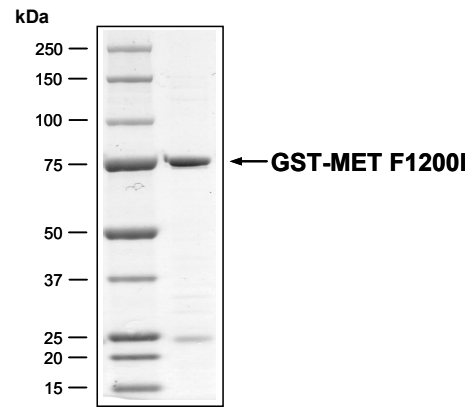
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 38 pmol/µg × min

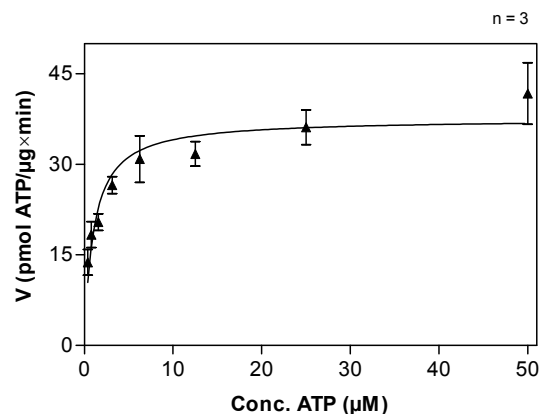
ATP-K<sub>M</sub>: 1.0 µM

### MET F1200I Lot 001: Coomassie stain



2.0 µg GST-MET F1200I

### MET F1200I Lot 001: 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 F1200I Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



# MET F1200I

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MET F1200I Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHH</b> HG	RRRASVAAGI	240
241	<b>LVPRGS</b> PGLD	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>VAKGM KYLAS</b>	<b>KKIVHRDLAA</b>	<b>RNCMLDEKFT</b>	<b>VKVADFGLAR</b>	<b>DMYDKEYYSV</b>	540
541	<b>HNKTGAKLPV</b>	<b>KWMALESLOT</b>	<b>QKFTTKSDVW</b>	<b>SFGVLLWELM</b>	<b>TRGAPPYPDV</b>	<b>NTFDITVYLL</b>	600
600	<b>QGRRLQPEY</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:** F1200I

MET wt <sup>1</sup> Amino Acid Sequence							
1	MKPAVLA PG	ILVLLFTLVQ	RSNGECKEAL	AKSEMNVNMK	YQLPNFTAET	PIQNVLHEH	60
61	HIFLGATNYI	YVLNEEDLQK	VAEYKTGPVL	EHPDCFPQD	CSSKANLSGG	VWKDNI NMAL	120
121	VVDTYDDQL	ISCGSVNRGT	CQRHVFPNH	TADIQSEVHC	IFSPQIEEPS	QCPDCVVSAL	180
181	GAKVLSSVKD	RFINFFVGNT	INSSYFPDHP	LHSISVRLK	ETKDGFMFLT	DQSYIDVLPE	240
241	FRDSYPIKYV	HAFESNNFIY	FLTQVRETLD	AQTFHTRIIR	FCSINSLGHS	YMEMPLECIL	300
301	TEKRKRSTK	KEVFNILQAA	YVSKPGAQLA	RQIGASLNDD	ILFGVFAQSK	PDSAEPMDRS	360
361	AMCAFPKIYV	NDFFNKIVNK	NNVRCLQHFY	GNHEHCENR	TLLRNSSGCE	ARRDEYRTEF	420
421	TTALQRVDLF	MGQFSEVLLT	SISTFIKGD L	TIANLGTSEG	RFMQVVVSRS	GPSTPHVNFL	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGKKI	TKIPLNGLGC	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQQI	CLPAIYK VFP	NSAPLEGGTR	LTICGWDFGF	RRNNKFDLKK	600
600	TRVLLGNESC	TLTLSESTMN	TLKCTVGPAM	NKHFNM SIII	SNGHGTTQYS	TFSYVDPVIT	660
661	SISPKYGPMA	GGTLLTLTGN	YLN SGN SRHI	SIGGKTCTLK	SVSNSILECY	TPAQTISTEF	720
721	AVKLIKIDLAN	RETSIFSYRE	DPIVYEIHPT	KSFISGGSTI	TGVGKNLNSV	SVPRMVINVH	780
781	EAGRNFTVAC	QHRSNSEIIC	CTTPSLQQLN	LQLPLKTKAF	FMLDGILSKY	FDLIYVHN PV	840
841	FKPFEKPVMI	SMGNENVLEI	KGNDIDPEAV	KGEVLKVGNK	SCENIHLHSE	AVLCTVPNDL	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>IGPSSLIVHF</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>GSPLVLPY M</b>	<b>KHGDLRNFIR</b>	<b>NETHNPTVKD</b>	<b>LIGFGLQVAK</b>	<b>GMKYLASKK<b>F</b></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>RLLOPEYCPD</b>	<b>PLYEVMLKCW</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)