

# Certificate of Analysis

## MET Y1235D

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0993-0000-1

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), Y1235D mutant, N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** MET Y1235D Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 78,739 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:** 0.347 µg/µl

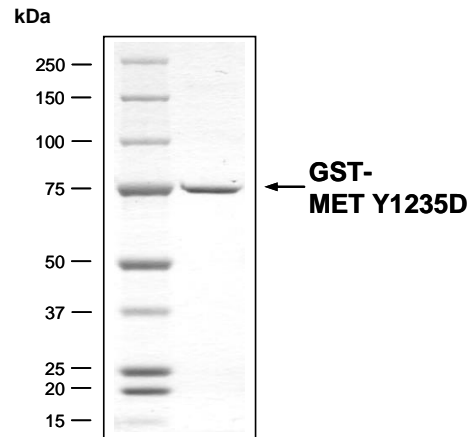
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 48 pmol/µg × min

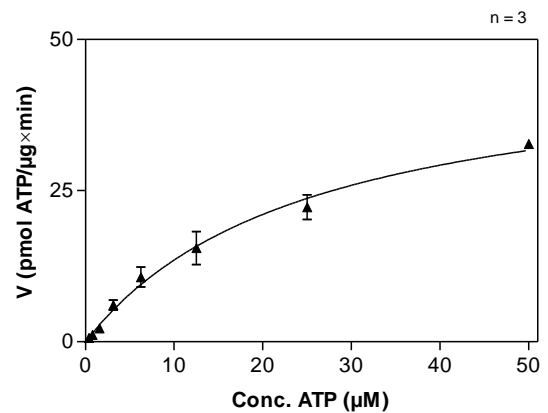
ATP-K<sub>M</sub>: 25 µM

**MET Y1235D Lot 001:**  
**Coomassie stain**



2.0 µg GST-MET Y1235D

**MET Y1235D 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 Y1235D 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 Y1235D

Product No.: 0993-0000-1

MET Y1235D 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	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSIEEFKK	RKQIKDLGSE	LVRVDARVHT	PHLDRLVSAR	SVSPTTEMVS	300
301	NESVDYRATF	PEDQFPNSSQ	NGSCRQVQYP	LTDMSPI LTS	GSDDISSPLL	QNTVHIDL SA	360
361	LNPELVQAVQ	HVIGPSSLI	VHFNEVIGRG	HFGCVYHGTL	LDNDGKKIHC	AVKSLNRITD	420
421	IGEVSQFLTE	GIIMKDFSHP	NVLSLLGICL	RSEGSPLVVL	PYMKHGDLRN	FIRNETHNPT	480
481	VKDLIGFGLQ	VAKGMKYLAS	KKFVHRDLAA	RNCMLDEKFT	VKVADFGLAR	DMYDKEYDSV	540
541	HNKTGAKLPV	KWMALESLOT	QKFTTKSDVW	SFGVLLWELM	TRGAPPYPDV	NTFDITVYLL	600
600	QGRRLQPEY	CPDPLYEVML	KCWHPKAEMR	PSFSELVSRI	SAIFSTFIGE	HYVHV NATYV	660
661	NVKCVAPYPS	LLSSEDNADD	EVDTRPASFW	ETS			720

1-218: GST    **Red:** HIS6-tag    **Pink:** Thrombin cleavage site    **blue:** MET fragment    **boxed:** Y1235D

MET wt <sup>1</sup> Amino Acid Sequence							
1	MKPAVAVLAPG	ILVLLFTLVQ	RSNGECKEAL	AKSEMNVNMK	YQLPNFTAET	PIQNVLVHEH	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	TIANLGTSEG	RFMQVVVRS	GPSTPHVNFL	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGKKI	TKIPLNGLGC	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQQI	CLPAIYKVP	NSAPLEGGTR	LTICGWDFGF	RRNNKFDLKK	600
600	TRVLLGNESC	TLTLESTMN	TLKCTVGPAM	NKHFNM SIII	SNGHGTTQYS	TFSYVDPVIT	660
661	SISPKYGPMA	GGTLLTLTGN	YLN SGN SRHI	SIGGKTCTLK	SVSNSILECY	TPAQTISTEF	720
721	AVKLIKIDLAN	RETSIFSYRE	DPIVYEIHPT	KSFISGGSTI	TGVGKNLNSV	SVPRMIVNH	780
781	EAGRNFTVAC	QHRSNSEIIC	CTTPSLQQLN	LQLPLKTKAF	FMLDGILSKY	FDLIYVHNPV	840
841	FKPFEKPVMI	SMGNENVLEI	KGNDIDPEAV	KGEVLKVGNK	SCENIHLHSE	AVLCTVPNDL	900
901	LKLNSELNIE	WKQAISSTVL	GKVIVQPDQN	FTGLIAGVVS	ISTALLLLLG	FFLWLKRRKQ	960
961	IKDLGSELVR	YDARVHTPHL	DRLVSARSVS	PTTEMVSNES	VDYRATFPED	QFPNSSQNGS	1020
1021	CRQVQYPLTD	MSPILTSGDS	DISSPLLQNT	VHIDL SALNP	ELVQAVQHVV	IGPSSLIVHF	1080
1081	NEVIGRGHFG	CVYHGTL LLDN	DGKKIHCAVK	SLNRITDIGE	VSQFLTEGII	MKDFSHPNVL	1140
1141	SLLGICLRSE	GSPLVLPY M	KHGDLRNFIR	NETHNPTVKD	LIGFGLQVAK	GMKYLASKKF	1200
1201	VHRDLAARNC	MLDEKFTVKV	ADFGLARDMY	DKEYYSVHNK	TGAKLPVKWM	ALESLOTQKF	1260
1261	TTKSDVWSFG	VLLWELMTRG	APPYPDVNTF	DITVYLLQGR	RLLQPEYCPD	PLYEVMLKCW	1320
1321	HPKAEMRPSF	SELVSRISAI	FSTFIGEHYV	HVNATYVNVK	CVAPYPSLLS	SEDNADDEVD	1380
1381	TRPASFWETS						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)