Certificate of Analysis

REACTION BIOLOGY

ProQinase™ MET L1195V

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 1651-0000-1

Lot: 001

Description: Human MET C-terminal fragment, amino acids K_{956}-S_{1390} (as in NCBI/Protein entry NP_000236.2), $L_{1195}V$ point mutant, N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET L1195V Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 78,773 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

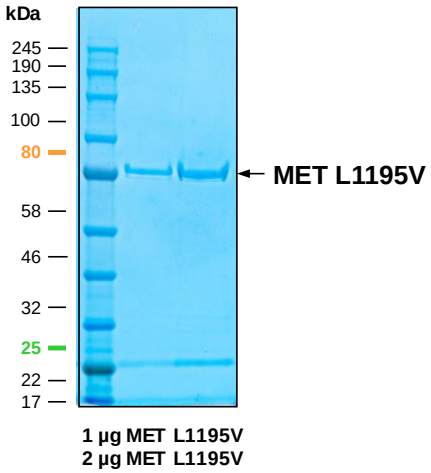
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

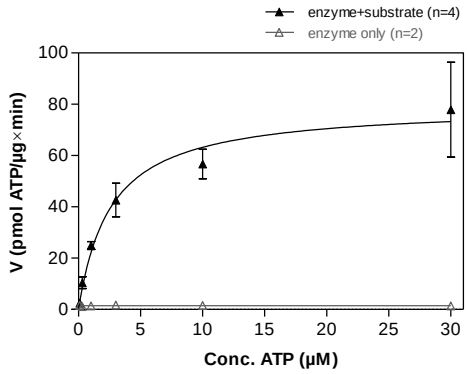
Protein concentration: 0.173 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 80 pmol/µg × min
ATP-K_M: 2.6 µM

MET L1195V Lot 001:
Coomassie stain



MET L1195V Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: TRK-C derived peptide 20 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
MET L1195V Lot 001 was also successfully tested by Reaction Biology 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

Recombinant Proteins

ProQinase™ MET L1195V

Product No.: 1651-0000-1

GST-MET L1195V Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
 241  LVPRGSPGLD GICSIEEFKK RKQIKDLGSE LVRYDARVHT PHLDRLVSAR SVSPTTEMVS 300
 301  NESVDYRATF PEDQFPNSSQ NGSCRQVQYP LTDMSPILTS GDSDISSPLL QNTVHIDLSA 360
 361  LNPELVQAVQ HVVIGPSSLI VHFNEVIGRG HFGCVYHGTL LDNDGKKIHC AVKSLNRITD 420
 421  IGEVSQFLTE GIIMKDFSHP NVLSLLGICL RSEGSPLVVL PYMKHGDLRN FIRNETHNPT 480
 481  VKDLIGFGLQ VAKGMKYVAS KKFVHRDLAA RNCMLDEKFT VKVADFGLAR DMYDKEYYSV 540
 541  HNKTGAKLPV KWMALESLQT QKFTTKSDVW SFGVLLWELM TRGAPPYPDV NTFDITVYLL 600
 601  QGRRLLQPEY CPDPLYEVML KCWHPKAEMR PSFSELVSRI SAIFSTFIGE HYVHVNATYV 660
 661  NVKCVAPYPS LLSSEDNADD EVDTRPASFW ETS                              720
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: MET fragment **boxed**: L1195V point mutation

MET wt[1] Amino Acid Sequence

```
1     MKAPAVLAPG ILVLLFTLVQ RSNGECKEAL AKSEMNVNMK YQLPNFTAET PIQNVILHEH  60
61    HIFLGATNYI YVLNEEDLQK VAEYKTGPVL EHPDCFPCQD CSSKANLSGG VWKDNINMAL 120
121   VVDTYYDDQL ISCGSVNRGT CQRHVFPHNH TADIQSEVHC IFSPQIEEPS QCPDCVVSAL 180
181   GAKVLSSVKD RFINFFVGNT INSSYFPDHP LHSISVRRLK ETKDGFMFLT DQSYIDVLPE 240
241   FRDSYPIKYV HAFESNNFIY FLTVQRETLD AQTFHTRIIR FCSINSGLHS YMEMPLECIL 300
301   TEKRKKRSTK KEVFNILQAA YVSKPGAQLA RQIGASLNDD ILFGVFAQSK PDSAEPMDRS 360
361   AMCAFPIKYV NDFFNKIVNK NNVRCLQHFY GPNHEHCFNR TLLRNSSGCE ARRDEYRTEF 420
421   TTALQRVDLF MGQFSEVLLT SISTFIKGDL TIANLGTSEG RFMQVVVSRS GPSTPHVNFL 480
481   LDSHPVSPEV IVEHTLNQNG YTLVITGKKI TKIPLNGLGC RHFQSCSQCL SAPPFVQCGW 540
541   CHDKCVRSEE CLSGTWTQQI CLPAIYKVFP NSAPLEGGTR LTICGWDFGF RRNNKFDLKK 600
600   TRVLLGNESC TLTLSESTMN TLKCTVGPAM NKHFNMSIII SNGHGTTQYS TFSYVDPVIT 660
661   SISPKYGPMA GGTLLTLTGN YLNSGNSRHI SIGGKTCTLK SVSNSILECY TPAQTISTEF 720
721   AVKLKIDLAN RETSIFSYRE DPIVYEIHPT KSFISGGSTI TGVGKNLNSV SVPRMVINVH 780
781   EAGRNFTVAC QHRSNSEIIC CTTPSLQQLN LQLPLKTKAF FMLDGILSKY FDLIYVHNPV 840
841   FKPFEKPVMI SMGNENVLEI KGNDIDPEAV KGEVLKVGNK SCENIHLHSE AVLCTVPNDL 900
901   LKLNSELNIE WKQAISSTVL GKVIVQPDQN FTGLIAGVVS ISTALLLLLG FFLWLKKRKQ 960
961   IKDLGSELVR YDARVHTPHL DRLVSARSVS PTTEMVSNES VDYRATFPED QFPNSSQNGS 1020
1021  CRQVQYPLTD MSPILTSGDS DISSPLLQNT VHIDLSALNP ELVQAVQHVV IGPSSLIVHF 1080
1081  NEVIGRGHFG CVYHGTLLDN DGKKIHCAVK SLNRITDIGE VSQFLTEGII MKDFSHPNVL 1140
1141  SLLGICLRSE GSPLVVLPYM KHGDLRNFIR NETHNPTVKD LIGFGLQVAK GMKYLASKKF 1200
1201  VHRDLAARNC MLDEKFTVKV ADFGLARDMY DKEYYSVHNK TGAKLPVKWM ALESLQTQKF 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 recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_000236.2

Please notice:
Variant amino acid numbering beginning with Ser755 when refering to GenBank accession number J02958 / NCBI/Protein accession number AAA59591.1 (additional 18 aa exon between S755/G756, frequently found in the literature)

Recombinant Proteins