

Certificate of Analysis

MET Y1230H

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0976-0000-1

Lot: 001

Description: Human MET C-terminal fragment, amino acids K₉₅₆-S₁₃₉₀ (as in NCBI/Protein entry NP_000236.2), Y1230H mutant, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET Y1230H Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 78,761 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: 0.295 µg/µl

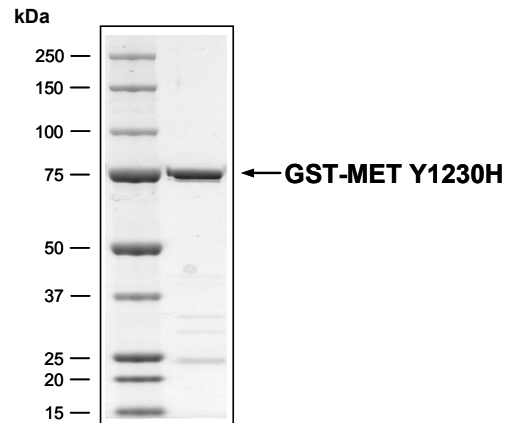
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 116 pmol/µg × min

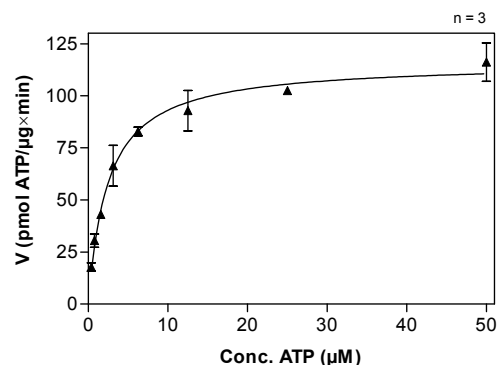
ATP-K_M: 2.5 µM

**MET Y1230H Lot 001:
Coomassie stain**



2.0 µg GST-MET Y1230H

**MET Y1230H 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 MgCl₂
 - 3 mM MnCl₂
 - 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: 1.0 µg/ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: MET Y1230H 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 Y1230H

Product No.: 0976-0000-1

| MET Y1230H 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 | PMGHHHHHGG | RRRASVAAGI | 240 |
| 241 | LVPRGSPGLD | GICSIEEFKK | RKQIKDLGSE | LVRVDARVHT | PHLDRLVSAR | SVSPTTEMVS | 300 |
| 301 | NEVDYRATF | PEDQFPNSSQ | NGSCRQVQYP | LTDMSPIILTS | GSDDISSPLL | QNTVHIDLSA | 360 |
| 361 | LNPELVQAVQ | HVIGPSSLI | VHFNEVIGRG | HFGCVYHGTL | LDNDGKKIHC | AVKSLNRITD | 420 |
| 421 | IGEVSQFLTE | GIIMKDFSHP | NVLSLLGICL | RSEGSPLVVL | PYMKHGDLRN | FIRNETHNPT | 480 |
| 481 | VKDLIGFGLQ | VAKGMKYLAS | KKFVHRDLAA | RNCMLDEKFT | VKVADFGLAR | DMHDKKEYYSV | 540 |
| 541 | HNKTGAKLPV | KWMALESLOT | QKFTTKSDVW | SFGVLLWELM | TRGAPPYPDV | NTFDITVYLL | 600 |
| 600 | QGRRLQPEY | 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:** Y1230H

| MET wt ¹ Amino Acid Sequence | | | | | | | |
|---|-------------|-------------|-------------|------------|------------|-------------|------|
| 1 | MKPAVAVLAPG | ILVLLFTLVQ | RSNGECKEAL | AKSEMNVNMK | YQLPNFTAET | PIQNVLILHEH | 60 |
| 61 | HIFLGATNYI | YVLNEEDLQK | VAEYKTGPVL | EHPDCFPQD | CSSKANLSGG | VWKDNIINMAL | 120 |
| 121 | VVDYYDDQL | ISCGSVNRGT | CQRHVFPNH | TADIQSEVHC | IFSPQIEEPS | QPCDCVVSAL | 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 | AMCAFPIKYV | 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 | NKHFNMIII | SNGHGTTQYS | TFSYVDPVIT | 660 |
| 661 | SISPKYGPMA | GGTLLTLTGN | YLNNSGNSRHI | SIGGKTCTLK | SVSNSILECY | TPAQTISTEF | 720 |
| 721 | AVKLIKIDLAN | RETSIFSIRE | DPIVYEIHT | KSFISGGSTI | TGVGKNLNSV | SVPRMIVNH | 780 |
| 781 | EAGRNFTVAC | QHRNSSEIIC | 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 | VHIDLSALNP | ELVQAVQHVV | IGPSSLIVHF | 1080 |
| 1081 | NEVIGRGHFG | CVYHGTLLEDN | DGKKIHCAVK | SLNRITDIGE | VSQFLTEGII | MKDFSHPNVL | 1140 |
| 1141 | SLLGICLRSE | GSPLVLPYM | KHGDLRNFIR | NETHNPTVKD | LIGFGLQVAK | GMKYLASKKF | 1200 |
| 1201 | VHRDLAARNC | MLDEKFTVKV | ADFGLARDMY | DKEYYSVHNC | 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

¹NCBI/Protein accession number NP_000236.2

Please notice:

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