

ProKinase™ MET Y1230D

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0981-0000-1

Lot: 001

Description: Human MET C-terminal fragment, amino acids K₉₅₆-S₁₃₉₀ (as in NCBI/Protein entry NP_000236.2), Y1230D mutant, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET Y1230D Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 78,727 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: 0.590 µg/µl

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

Biochemical Parameters:

Specific kinase activity (P_i transfer): 222 pmol/µg × min

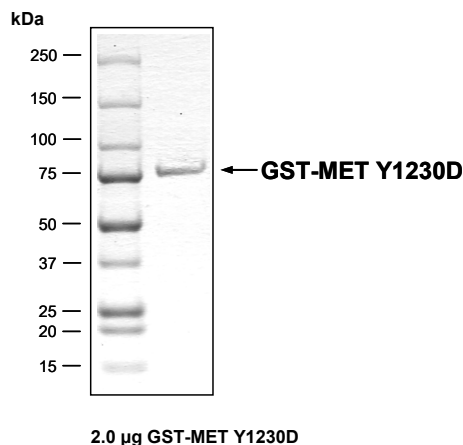
ATP-K_M: 5.2 µM

Additional assay technology: MET Y1230D Lot 001

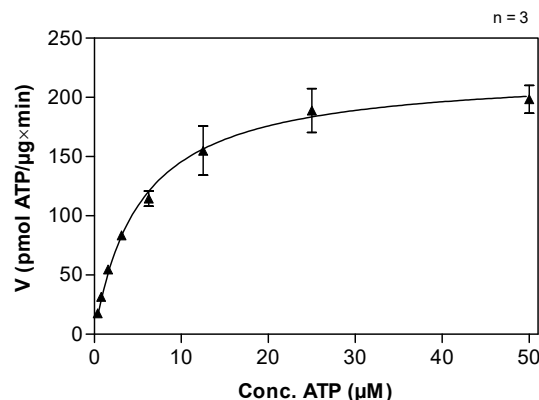
was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



MET Y1230D Lot 001:
Coomassie stain



MET Y1230D 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)

Recombinant Proteins

ProQinase™ MET Y1230D

Product No.: 0981-0000-1

| MET Y1230D Recombinant Fusion Protein Amino Acid Sequence | | | | | | | |
|---|-------------|------------|------------|-------------|------------|-------------|-----|
| 1 | MSPILGYWKI | KGLVQPTRLL | LEYLEEKYEE | HLYERDEGDK | WRNKKFELGL | EFPNLPYYID | 60 |
| 61 | GDVKLTQSM | IIRYIADKHN | MLGGCPKERA | EISMLEGAVL | DIRYGVSRIA | YSKDFETLKV | 120 |
| 121 | DFLSKLP EML | KMFEDRLCHK | TYLNGDHVTH | PDFMLYDALD | VVLYMDPMCL | DAFPKLVCFK | 180 |
| 181 | KRIEAI PQID | KYLKSSKYIA | WPLQGWQATF | GGGDHPPKSD | PMGHHHHHGG | RRRASVAAGI | 240 |
| 241 | LVPRGSPGLD | GICSIEEFKK | RKQIKDLGSE | LVRYDARVHT | PHLDRLVSAR | SVSPTTEMVS | 300 |
| 301 | NESVDYRATF | PEDQFPNSSQ | NGSCRQVQYP | LTDMSPIILTS | GSDSISSPLL | 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 | DMDDKEYYSV | 540 |
| 541 | HNKTGAKLPEY | KWMALESLOT | QKFTTKSDVW | SFGVLLWELM | TRGAPPYPDV | NTFDITVYLL | 600 |
| 600 | QGRRLLOPEY | CPDPLYEVML | KCWHPKAEMR | SFSELVSRI | SAIFSTFIGE | HYVHV NATYV | 660 |
| 661 | NVKCVAPYPS | LLSSEDNADD | EVDTRPASFW | ETS | | | 720 |

1-218: GST Red: HIS6-tag Pink: Thrombin cleavage site blue: MET fragment boxed: Y1230D

| MET wt ¹ Amino Acid Sequence | | | | | | | |
|---|-------------|-------------|--------------|-------------|------------|-------------|------|
| 1 | MKAPAVLAPG | ILVLLFTLVQ | RSNGECKEAL | AKSEMNVNMK | YQLPNFTAET | PIQNVILHEH | 60 |
| 61 | HIFLGATNYI | YVLNEEDLQK | VAEYKTGPVL | EHPDCFPQD | CSSKANLGG | VWKDNINMAL | 120 |
| 121 | VVDYYDDQL | ISCGSVNRGT | CQRHVFPNH | TADIQSEVHC | IFSPQIEEPS | QCPDCVVSAL | 180 |
| 181 | GAKVLSSVKD | RFINFFVGNT | INSSYFPDHP | LHSISVRRLK | ETKDGFMFLT | DQSYIDL VPE | 240 |
| 241 | FRDSYPIKYV | HAFESNNFIY | FLTVQRETLD | AQTFHTRIIR | FCSINSLGHS | YMEMPLECIL | 300 |
| 301 | TEKRKKRSTK | KEVFNILQAA | YVSKPGAQLA | RQIGASLNDD | ILFGVFAQSK | PDSAEPMDRS | 360 |
| 361 | AMCAFFPIKYV | NDFFNKIVNK | NNVRCLQHFY | GNHEHC FNR | TLLRNSSGCE | ARRDEYRTEF | 420 |
| 421 | TTALQRVDLF | MGQFSEVLLT | SISTFIKGD | TIANLGTSEG | RFMQVVVRS | GPSTPHVNFL | 480 |
| 481 | LDSHPVSPEV | IVEHTLNQNG | YTLVITGKKI | TKIPLNGLGC | RHFQSCSQCL | SAPPFVQCGW | 540 |
| 541 | CHDKCVRSEE | CLSGTWTQQI | CLPAIYKVPF | NSAPLEGGTR | LTICGWDFGF | RRNNKFDLKK | 600 |
| 600 | TRVLLGNESE | TLTLESTMN | TLKCTVGPAM | NKHFNMSIII | SNGHGTTQYS | TFSYVDPVIT | 660 |
| 661 | SISPKYGPMA | GGTLLTLTGN | YLN SGN SRHI | SIGGKTCTLK | SVSNSILECY | TPAQTISTEF | 720 |
| 721 | AVKLIKIDLAN | RETSIFSYRE | DPIVYEIHPT | KSFISGGSTI | TGVGKNLNSV | SVPRMVINVH | 780 |
| 781 | EAGRNFVAC | QHRNSEIIC | CTTPSLQQLN | LQLPLKTKAF | FMLDGILSKY | FDLIYVHNPV | 840 |
| 841 | FKPFEKPVMI | SMGNENVLEI | KGNDIDPEAV | KGEVLKVGNK | SCENIHLHSE | AVLCTVPNDL | 900 |
| 901 | LKLNSELNIE | WKQAISSTVL | GKVIVQPDQN | FTGLIAGVVS | ISTALLLLL | FFLWLKKRKQ | 960 |
| 961 | IKDLGSELVR | YDARVHTPHL | DRLVSARSVS | PTEMVSNES | VDYRATFPED | QFPNSSQNGS | 1020 |
| 1021 | CRQVQYPLTD | MSPILTS GDS | DISSPLLQNT | VHIDL SALNP | ELVQAVQHV | IGPSSLIVHF | 1080 |
| 1081 | NEVIGRGHFG | CVYHGTL LDN | DGKKIHCAVK | SLNRITDIGE | VSQFLTEGII | MKDFSHPNVL | 1140 |
| 1141 | SLLGICLRSE | GSPLVLPYM | KHGDLRNFIR | NETHNPTVKD | LIGFGLQVAK | GMYLASKKF | 1200 |
| 1201 | VHRDLAARNC | MLDEKFTVKV | ADFG LARDMY | DKEYYSVH NK | TGAKLPVKWM | ALESLOTQKF | 1260 |
| 1261 | TTKSDVWSFG | VLLWELMTRG | APPYPDVNTF | DITVYLLQGR | RLLQPEYCPD | PLYEVMLKCW | 1320 |
| 1321 | HPKAEMRPSF | SELVSRI SAI | FSTFIGEHYV | HVNATYVNVK | CVAPYPSLLS | SEDNADDEV | 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 numbering beginning with Ser755 when referring to GenBank accession J02958 (additional 18 aa exon between S755/G756, frequently found in the literature)