

MET Y1230A

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0944-0000-1

Lot: 002

Description: Human MET C-terminal fragment, amino acids K₉₅₆-S₁₃₉₀ (as in NCBI/Protein entry NP_000236.2), Y1230A mutant, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET Y1230A Lot 002 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 78,695 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: 0.421 µg/µl

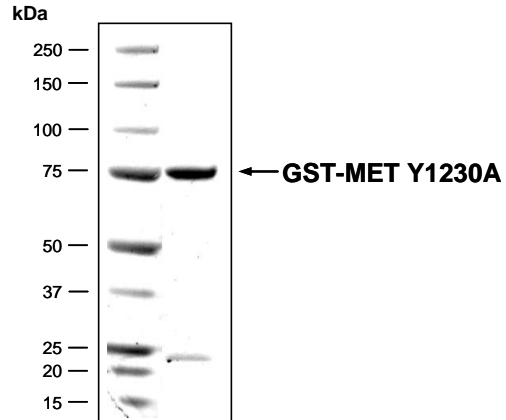
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 106 pmol/µg × min

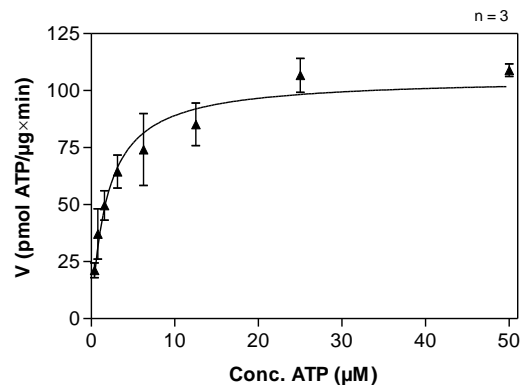
ATP-K_M: 1.9 µM

MET Y1230A Lot 002:
Coomassie stain



2.0 µg GST-MET Y1230A

MET Y1230A Lot 002:
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 Y1230A Lot 002

was also successfully tested by ProQinase 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



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MET Y1230A 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 HHHHHH HG	RRRASVAAGI	240
241	LVPRG SPGLD	GICSIEEFKK	RKQIKDLGSE	LVRVDARVHT	PHLDRLVSAR	SVSPTTEMVS	300
301	NESVDYRATF	PEDQFPNSSQ	NGSCRQVQYP	LTDMSPI LTS	GSDDISSPLL	QNTVHIDLSA	360
361	LNPELVQAVQ	HVVI GPSSLI	VHFNEVIGRG	HFGCVYHGTL	LDNDGKKIHC	AVKSLNRITD	420
421	IGEVSOFLTE	GIIMKDFSHP	NVLSLLGICL	RSEGSPLVVL	PYMKHGDLRN	FIRNETHNPT	480
481	VKDLIGFGLQ	VAKGMKYLAS	KKFVHRDLAA	RNCMLDEKFT	VKVADFGLAR	DMADKEYYSV	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:** Y1230A

MET wt ¹ 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	FLTQVRETL	AQTFHTRIIR	FCSINSLGHS	YMEMPLECIL	300
301	TEKRKRSTK	KEVFNIIQAA	YVSKPGAQLA	RQIGASLNDD	ILFGVFAQSK	PDSAEPMDRS	360
361	AMCAFPKIYV	NDFFNKIVNK	NNVRCLQHFY	GNHEHCENR	TLLRNSSGCE	ARRDEYRTEF	420
421	TTALQRVDLF	MGQFSEVLLT	SISTFIKGD	TIANLGTSEG	RFMQVVVSR	GPSTPHVNFL	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGKKI	TKIPLNGLGC	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQOI	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	FFLWL KKRKQ	960
961	IKDLGSELVR	YDARVHTPHL	DRLVSARSVS	PTTEMVSNES	VDYRATFPED	QFPNSSQNGS	1020
1021	CRQVQYPLTD	MSPILTS GDS	DISSPLLQNT	VHIDLSALNP	ELVQAVQHVV	IGPSSLIVHF	1080
1081	NEVIGRGHFG	CVYHGTL LDN	DGKKIHCAVK	SLNRITDIGE	VSQFLTEGII	MKDFSHPNVL	1140
1141	SLLGICLRSE	GSPLVLPYM	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

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)