Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ MET D1228N

MET proto-oncogene, receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0994-0000-1

Lot: 001

Description: Human MET C-terminal fragment, amino acids K_{956}-S_{1390} (as in NCBI/Protein entry NP_000236.2), D1228N mutant, N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET D1228N Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{\text{Fusion Protein}}$: 78,787 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

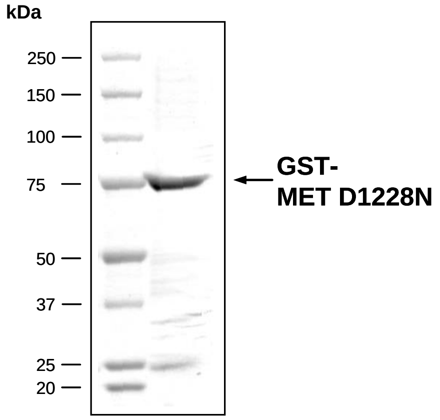
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

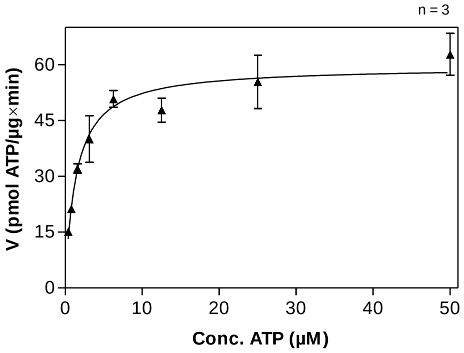
Biochemical Parameters:
Specific kinase activity (P_i transfer): 59 pmol/µg × min
ATP-K_M: 1.4 µM

MET D1228N Lot 001:
Coomassie stain



2.0 µg GST-MET D1228N

MET D1228N 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 D1228N Lot 001 was also successfully tested by Reaction Biology for the use with the ADP-Glo^{TM} Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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ProQinase™ MET D1228N

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GST-MET D1228N 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	PMG**HHHHHH**G	RRRASVAAGI	240
241	**LVPRGS**PGLD	GICSIEEF**KK**	**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**	**VAKGMKYLAS**	**KKFVHRDLAA**	**RNCMLDEKFT**	**VKVADFGLAR**	**NMYDKEYYSV**	540
541	**HNKTGAKLPV**	**KWMALESLQT**	**QKFTTKSDVW**	**SFGVLLWELM**	**TRGAPPYPDV**	**NTFDITVYLL**	600
600	**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**: D1228N

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	FFLWL**KKRKQ**	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 J02958 (additional 18 aa exon between S755/G756, frequently found in the literature)

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Version 001