Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ KIT V560G

KIT proto-oncogene, receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCFR, c-Kit

Product No.: 1046-0000-1

Lot: 002

Description: Human KIT, C-terminal fragment, amino acids T_{544}-V_{976} (as in NCBI/Protein entry NP_000213.1) with a V560G mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: KIT V560G Lot 002 was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 77,415 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

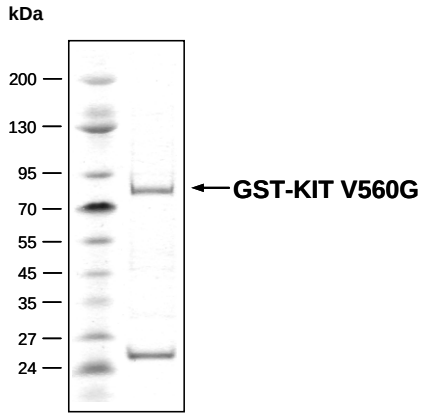
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

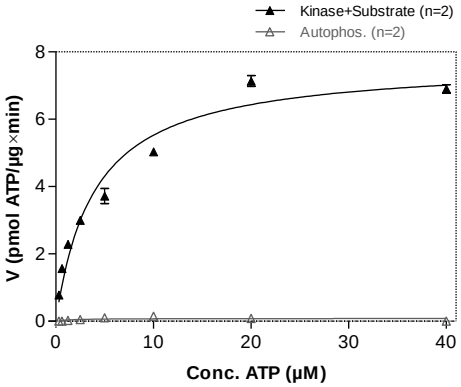
Biochemical Parameters:
Specific kinase activity (P_i transfer): 8 pmol/µg × min
ATP-K_M: 3.9 µM

KIT V560G Lot 002:
Coomassie stain



2.0 µg GST-KIT V560G

KIT V560G Lot 002:
Determination of V_{max} and K_M value for ATP



• 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 80 µg/ml
Kinase: 4 µg/ml
• Filter binding assay
MSPH membrane (Millipore)

Additional assay technology:
KIT V560G Lot 002 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

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GST-KIT V560G Recombinant Fusion Protein Amino Acid Sequence

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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 RDSLEVLFQG  240
241   PLAMGTYKYL QKPMYEVQWK VGEEINGNNY VYIDPTQLPY DHKWEFPRNR LSFGKTLGAG  300
301   AFGKVVEATA YGLIKSDAAM TVAVKMLKPS AHLTEREALM SELKVLSYLG NHMNIVNLLG  360
361   ACTIGGPTLV ITEYCCYGDL LNFLRRKRDS FICSKQEDHA EAALYKNLLH SKESSCSDST  420
421   NEYMDMKPGV SYVVPTKADK RRSVRIGSYI ERDVTPAIME DDELALDLED LLSFSYQVAK  480
481   GMAFLASKNC IHRDLAARNI LLTHGRITKI CDFGLARDIK NDSNYVVKGN ARLPVKWMAP  540
541   ESIFNCVYTF ESDVWSYGIF LWELFSLGSS PYPGMPVDSK FYKMIKEGFR MLSPEHAPAE  600
601   MYDIMKTCWD ADPLKRPTFK QIVQLIEKQI SESTNHIYSN LANCSPNRQK PVVDHSVRIN  660
661   SVGSTASSSQ PLLVHDDV                                               720
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: KIT fragment **boxed**: V560G

KIT wt[1] Amino Acid Sequence

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1     MRGARGAWDF LCVLLLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD  60
61    PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV  120
121   DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH  180
181   RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS  240
241   SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN  300
301   VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE  360
361   DYPKSENESN IRYVSELHLT RLKGTEGGTY TFLVSNSDVN AAIAFNVYVN TKPEILTYDR  420
421   LVNGMLQCVA AGFPEPTIDW YFCPGTEQRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS  480
481   SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV  540
541   MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF  600
600   GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC  660
661   TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE  720
721   YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM  780
781   AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES  840
841   IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY  900
901   DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV  960
961   GSTASSSQPL LVHDDV                                                 1020
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blue: KIT sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_000213.1

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