

ProQinase™ KIT V559D/T670I

v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCFR, c-Kit

Product No.: 1044-0000-1

Lot: 005

Description: Human KIT, C-terminal fragment, amino acids T₅₄₄-V₉₇₆ (as in NCBI/Protein entry NP_000213.1) with a V559D and a T670I mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: KIT V559D/T670I Lot 002 was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 77,485 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: 0.109 µg/µl

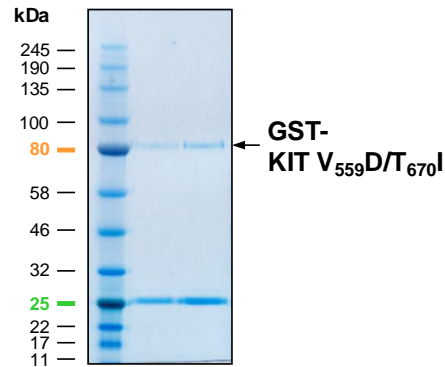
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 6 pmol/µg × min

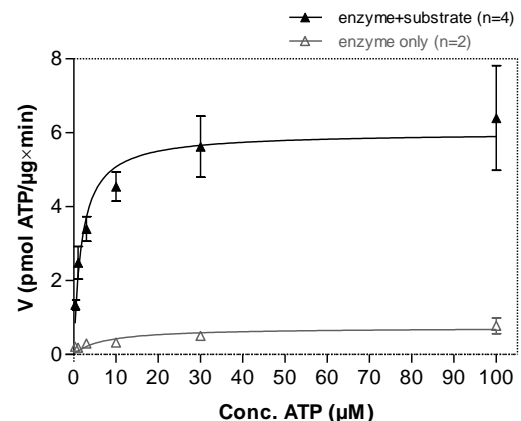
ATP-K_M: 1.8 µM

KIT V559D/T670I LOT005:
Coomassie stain



1 µg GST-KIT V559D/T670I
2 µg GST-KIT V559D/T670I

KIT V559D/T670I LOT005:
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 80 µg/ml
 - Kinase: 4 µg/ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: KIT V559D/T670I LOT005

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



ProQinase™ KIT V559D/T670I

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GST- KIT V559D/T670I Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGWYKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLQTSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQG WQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVL FQG	240
241	PLAMGTYKYL	QKPMYEVQWK	DVEEINGNNY	VYIDPTQLPY	DHKWEFPRNR	LSFGKTLGAG	300
301	AFGKVV EATA	YGLIKSDAAM	TVAVKMLKPS	AHLTEREALM	SELKVL SYLG	NHMNIVNLLG	360
361	ACTIGGPTLV	IIEYCCYGDL	LNFLRRKRDS	FICSKQEDHA	EAALYKNLLH	SKESSCSDST	420
421	NEYMDMKPGV	SYVVP TKADK	RRSVRIGSYI	ERDVT PAIME	DDELALDLED	LLSFSYQVAK	480
481	GMAFLASKNC	IHRDLAARNI	LLTHGRITKI	CDFGLARDIK	NDSNYVVKGN	ARLPVKWMAP	540
541	ESIFNCVYTF	ESDVWSYGIF	LWELFSLGSS	PYPGMPVDSK	FYKMIKEGFR	MLSPEHAPAE	600
601	MYDIMKTCDW	ADPLKRPTFK	QIVQLIEKQI	SESTNHIYSN	LANCSPNRQK	PVVDHSVRIN	660
661	SVGSTASSSQ	PLLVHDDV					720

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: KIT fragment boxed: V559D/T670I mutations

KIT wt ¹ Amino Acid Sequence							
1	MARGAWDF	LCVLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDETENKQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKFLV	120
121	DRSLYGKEDN	DTLVRCP L TD	PEVTNYS L KG	CQ GKPLPKDL	RFIPDPKAGI	MIKSVKRAYH	180
181	RLCLHCSVDQ	EGKSVLSEKF	ILKVRPAFKA	VPVSVSKAS	YLLREG E EFT	VTCTIKDVSS	240
241	SVYSTWKREN	SQTKLQEKYN	SWHHGDFNYE	RQATLT I SSA	RVNDSGVFMC	YANNTFGSAN	300
301	VTTTLEVVDK	GFINIFPMIN	TTV FVNDGEN	VDLIVEYEAF	PKPEHQQWIY	MNRTFTDKWE	360
361	DYPKSENE SN	IRYVSELH LT	RLKGTEGGTY	TFLVSN SDVN	AAIAFN VYVN	TKPEILTYDR	420
421	LVNGMLQCVA	AGFPEPTIDW	YFCPGTEQRC	SASVLPVDVQ	TLNSSGPPFG	KLVVQSSIDS	480
481	SAFKHNGTVE	CKAYNDVGKT	SAYFNFAFKG	NNKEQIHPHT	LFTPLLIGFV	IVAGMMCIIV	540
541	MILTYKYLQK	PMEVQWKVV	EEINGN NYVY	IDPTQLPYDH	KWEFPRNRLS	FGKTLGAGAF	600
600	GKVV EATAYG	LIKSDAAMTV	AVKMLKPSAH	LTEREALMSE	LKVL SYLGNH	MNIVNLLGAC	660
661	TIGGPTLVIT	EYCCYGDLLN	FLRRKRDSFI	CSKQEDHAEA	ALYKNLLH SK	ESSCSDSTNE	720
721	YMDMKPGVSY	VVPTKADKRR	SVRIGSYIER	DVTPAIME DD	ELALDLEDLL	SFSYQVAKGM	780
781	AFLASKNCIH	RDLAARN ILL	THGRITKICD	FGLARDIKND	SNYVVKGNAR	LPVKWMAPE S	840
841	IFNCVYTFES	DVWSYGIFLW	ELFSLGSSPY	PGMPVDSK FY	KMIKEGFRML	SPEHAPAEMY	900
901	DIMKTCDWAD	PLKRPTFKQI	VQLIEKQISE	STNHIYSNLA	NCSPNRQKPV	VDHSVRINSV	960
961	GSTASSSQPL	LVHDDV					1020

blue: KIT sequence expressed in recombinant protein Red: variant in recombinant protein

¹NCBI/Protein accession number NP_000213.1

Recombinant Proteins