

## ProQinase™ KIT V559D

KIT proto-oncogene receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCFR, c-Kit

Product No.: 1047-0000-1

Lot: 003

**Description:** Human KIT, C-terminal fragment, amino acids T<sub>544</sub>-V<sub>976</sub> (as in [NCBI/Protein](#) entry NP\_000213.1) with a V<sub>559</sub>D mutation, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** KIT V559D Lot 003 was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 77,473 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:** 0.174 µg/µl

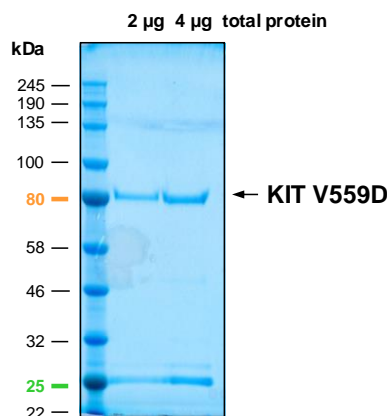
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

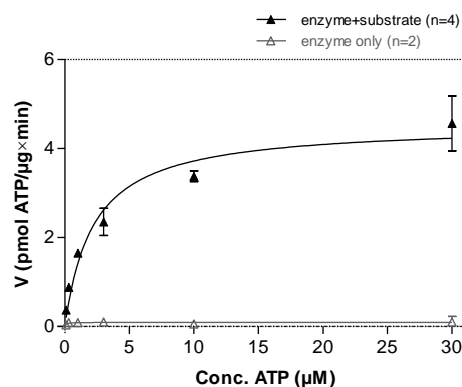
Specific kinase activity (P<sub>i</sub> transfer): 4.5 pmol/µg × min

ATP-K<sub>M</sub>: 2.2 µM

**KIT V559D Lot 003:**  
Coomassie stain



**KIT V559D Lot 003:**  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



- Assay conditions:  
60 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 µM Na-orthovanadate  
1.2 mM DTT  
50 µg/ml PEG<sub>20,000</sub>  
ATP (variable)  
Substrate: TRK-C derived peptide 5 µg/ml  
Kinase: 2 µg/ml
- Filter binding assay  
MSIP membrane (Millipore)

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GST-KIT V559D 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	WPLQG WQATF	GGGDHPPKSD	PMG <b>HHHHHG</b>	RDS <b>LEVLFQG</b>	240
241	<b>PLAMGTYKYL</b>	<b>QKPMYEVQWK</b>	<b>DVEEINGNNY</b>	<b>VYIDPTQLPY</b>	<b>DKHWEFPRNR</b>	<b>LSFGKTLGAG</b>	300
301	<b>AFGKVVEATA</b>	<b>YGLIKSDAAM</b>	<b>TVAVKMLKPS</b>	<b>AHLTEREALM</b>	<b>SELKVL SYLG</b>	<b>NHMNIVNLLG</b>	360
361	<b>ACTIGGPTLV</b>	<b>ITEYCCYGDL</b>	<b>LNFLRRKRDS</b>	<b>FICSKQEDHA</b>	<b>EAALYKNLLH</b>	<b>SKESSCSDST</b>	420
421	<b>NEYMDMKPGV</b>	<b>SYVVP TKADK</b>	<b>RRSVRIGSYI</b>	<b>ERDVT PAIME</b>	<b>DDELALDLED</b>	<b>LLSFSYQVAK</b>	480
481	<b>GMAFLASKNC</b>	<b>IHRDLAARNI</b>	<b>LLTHGRITKI</b>	<b>CDFGLARDIK</b>	<b>NDSNYVVKGN</b>	<b>ARLPVKWMAP</b>	540
541	<b>ESIFNCVYTF</b>	<b>ESDVWSYGIF</b>	<b>LWELFSLGSS</b>	<b>PYPGMPVDSK</b>	<b>FYKMIKEGFR</b>	<b>MLSPEHAPAE</b>	600
601	<b>MYDIMKTCWD</b>	<b>ADPLKRPTFK</b>	<b>QIVQLIEKQI</b>	<b>SESTNHIYSN</b>	<b>LANCSPNRQK</b>	<b>PVVDHSVRIN</b>	660
661	<b>SVGSTASSSQ</b>	<b>PLLVHDDV</b>					720

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: KIT fragment **boxed**: V559D point mutation

KIT wt <sup>1</sup> Amino Acid Sequence							
1	MRGARGAWDF	LCVLLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDET NENKQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKFLFV	120
121	DRSLY GKEDN	DTLVR CPLTD	PEVTNYS LKG	CQ GKPLPKDL	RFIPDPKAGI	MIKSVKRAYH	180
181	RLCLHCSVDQ	EGKSVLSEKF	ILKVRPAFKA	VPVSVSKAS	YLLREG EEF T	VTCTIKDVSS	240
241	SVYSTWKREN	SQTKLQEKYN	SWHHGDFNYE	RQATLTISSA	RVNDSGVFMC	YANNTFGSAN	300
301	VTTTLEVVDK	GFINIFPMIN	TTVFVNDGEN	VDLIVEYEAF	PKPEHQQWIY	MNRTFTDKWE	360
361	DYPKSENE SN	IRYVSELHLT	RLKGTEGGTY	TFLVSNSDVN	AAIAFN VYVN	TKPEILTYDR	420
421	LVNGMLQCVA	AGFPEPTIDW	YFCPGTEQRC	SASVLPVDVQ	TLNSSGPPFG	KLVVQSSIDS	480
481	SAFKHNGTVE	CKAYNDVGKT	SAYFNFAFKG	NNKEQIHPHT	LFTPLLIGFV	IVAGMMCIIV	540
541	<b>MILTYKYLQK</b>	<b>PMYEVQWKV</b>	<b>EEINGN NYVY</b>	<b>IDPTQLPYDH</b>	<b>KWEFPRNRLS</b>	<b>FGKTLGAGAF</b>	600
600	<b>GKVVEATAYG</b>	<b>LIKSDAAMTV</b>	<b>AVKMLKPSAH</b>	<b>LTEREALMSE</b>	<b>LKVL SYLGNH</b>	<b>MNIVNLLGAC</b>	660
661	<b>TIGGPTLVIT</b>	<b>EYCCYGDLLN</b>	<b>FLRRKRDSFI</b>	<b>CSKQEDHAEA</b>	<b>ALYKNLLHSK</b>	<b>ESSCSDSTNE</b>	720
721	<b>YMDMKPGVSY</b>	<b>VVPTKADKRR</b>	<b>SVRIGSYIER</b>	<b>DVTPAIME DD</b>	<b>ELALDLEDLL</b>	<b>SFSYQVAKGM</b>	780
781	<b>AFLASKNCIH</b>	<b>RDLAARNIL</b>	<b>THGRITKICD</b>	<b>FGLARDIKND</b>	<b>SNYVVKGNAR</b>	<b>LPVKWMAPE S</b>	840
841	<b>IFNCVYTFES</b>	<b>DVWSYGI FLW</b>	<b>ELFSLGSSPY</b>	<b>PGMPVDSK FY</b>	<b>KMIKEGFRML</b>	<b>SPEHAPAEM Y</b>	900
901	<b>DIMKTCWDAD</b>	<b>PLKRPTFKQI</b>	<b>VQLIEKQISE</b>	<b>STNHIYSNLA</b>	<b>NCSPNRQKPV</b>	<b>VDHSVRINSV</b>	960
961	<b>GSTASSQPL</b>	<b>L VHDDV</b>					1020

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

<sup>1</sup>[NCBI/Protein](#) accession number NP\_000213.1