

# Certificate of Analysis

## KIT V559D/T670I

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

**Recombinant Human Active Protein Kinase**

**Synonyms:** CD117, PBT, SCFR, c-Kit

**Product No.:** 1044-0000-1

**Lot:** 002

**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 V559D and a T670I mutation, N-terminal GST-HIS<sub>6</sub> 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**<sub>Fusion Protein</sub>: 77,485 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** GST-Affinity Chromatography

**Activation:** in vitro autoactivation

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

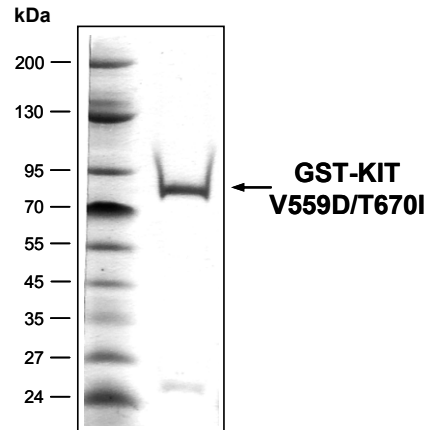
**Storage temperature:** -80°C  
Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.162 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

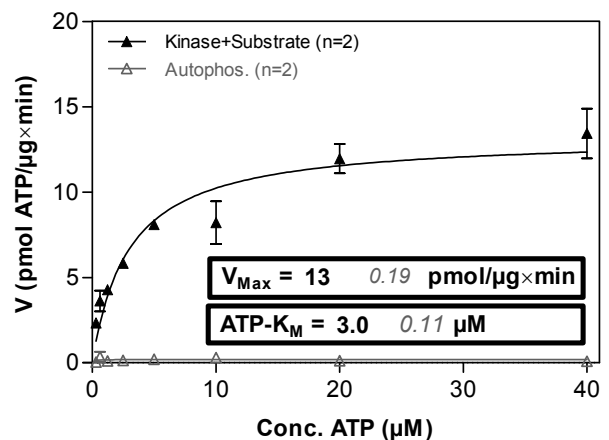
**Biochemical Parameters:**

Specific activity: 13 pmol/µg×min  
ATP-K<sub>M</sub>: 3.0 µM

**KIT V559D/T670I Lot 002:  
Coomassie stain**



**KIT V559D/T670I Lot 002:  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP**



**Determination of K<sub>M</sub> value & Specific activity:**

- 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, 80 µg / ml
  - KIT V559D/T670I: 4.0 µg / ml
- Filter binding assay
  - MSPH membrane (Millipore)

**Additional assay technology:** KIT V559D/T670I Lot 002

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from



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## KIT V559D/T670I

Product No.: 1044-0000-1

KIT V559D/T670I Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RDSLEVL FQG	240
241	PLAMGTYKYL	QKPMYEVQWK	DVEEINGNNY	VYIDPTQLPY	DHKWEFPRNR	LSFGKTLGAG	300
301	AFGKVV EATA	YGLIKSDAAM	TVAVKMLKPS	AHLTEREALM	SELKVL SYLG	NHMNI VNLG	360
361	ACTIGGPTLV	IIEYCCY GDL	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	MYDIMKTCWD	ADPLKRPTFK	QIVQLIEKQI	SESTNHIYSN	LANCSPNRQK	PVVDHSVRIN	660
661	SVGSTASSSQ	PLLHDDV					720

1-218: GST    Red: HIS6-tag    Green: 3C    blue: KIT fragment    D/I: V559D&T670I mutation

KIT wt <sup>1</sup> amino acid sequence							
1	MARGAWDF	LCVLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDET NENKQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKLFLV	120
121	DRSLYGKEDN	DTLVRCPLTD	PEVTNYSLKG	CQ GKPLPKDL	RFIPDPKAGI	MIKSVKRAYH	180
181	RLCLHCSVDQ	EGKSVLSEKF	ILKVRPAFKA	VPVSVSKAS	YLLREG EEF	VTCTIKDVSS	240
241	SVYSTWKREN	SQTKLQEKYN	SWHHGDFNYE	RQATLTISSA	RVNDSGVFMC	YANNTFGSAN	300
301	VTTTLEVVDK	GFINIFPMIN	TTVFVNDGEN	VDLIVEYEAF	PKPEHQWYI	MNRTFTDKWE	360
361	DYPKSENESEN	IRYVSELHLT	RLKGTEGGTY	TFLVSNSDVN	AAIAFN VYVN	TKPEILTYDR	420
421	LVNGMLQCVA	AGFPEPTIDW	YFCPGTEQRC	SASVLPVDVQ	TLNSSGPPFG	KL VVQSSIDS	480
481	SAFKHNGTVE	CKAYNDVGKT	SAYFNFAFKG	NNKEQIHPHT	LFTPLLIGFV	IVAGMMCIIV	540
541	MILTYKYLQK	PMYEVQWKVV	EEINGNNYVY	IDPTQLPYDH	KWEFPRNRLS	FGKTLGAGAF	600
600	GKVV EATAYG	LIKSDAAMTV	AVKMLKPSAH	LTEREALMSE	LKVL SYLGNH	MNI VNLGAC	660
661	TIGGPTLVIT	EYCCYDLLN	FLRRKRDSFI	CSKQEDHAEA	ALYKNLLHSK	ESSCSDSTNE	720
721	YMDMKPGVSY	VVPTKADKRR	SVRIGSYIER	DVT PAIMEDD	ELALDLEDLL	SFSYQVAKGM	780
781	AFLASKNCIH	RDLAARNILL	THGRITKICD	FGLARDIKND	SNYVVKGNAR	LPVKWMAPES	840
841	IFNCVYTFES	DVWSYGIFLW	ELFSLGSSPY	PGMPVDSKPY	KMIKEGFRML	SPEHAPAEMY	900
901	DIMKTCWDAD	PLKRPTFKQI	VQLIEKQISE	STNHIYSNLA	NCSPNRQKPV	VDHSVRINSV	960
961	GSTASSOPL	LHDDV					1020

bold letters: expressed part of KIT    RED letters: variant in Fusionprotein

<sup>1</sup>NCBI/Protein accession number NP\_000213.1