

## ProQinase™ KIT V559D/V654A

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

**Recombinant Human Active Protein Kinase**

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

**Product No.:** 1045-0000-1

**Lot:** 001

**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 V654A mutation, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** KIT V559D/V654A Lot 001 was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 77,445 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

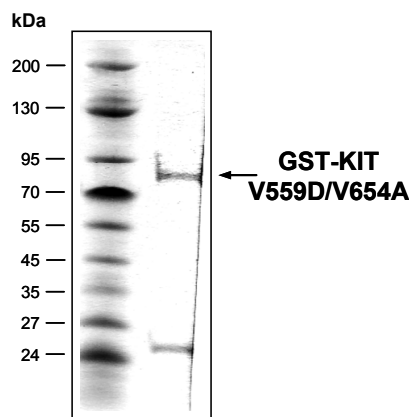
**Storage temperature:** -80°C

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

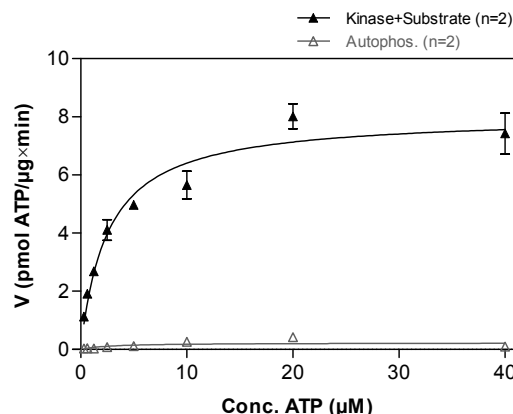
**Biochemical Parameters:**  
Specific kinase activity (P<sub>i</sub> transfer): 8 pmol/µg×min  
ATP-K<sub>M</sub>: 2.6 µM

**KIT V559D/V654A Lot 001:**  
Coomassie stain



2.0 µg GST-KIT V559D/V654A

**KIT V559D/V654A Lot 001:**  
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/V654A: 4.0 µg/ml
- Filter binding assay
  - MSPH membrane (Millipore)

**Additional assay technology:** KIT V559D/V654A Lot 001 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/V654A

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KIT V559D/V654A Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFG	240
241	PLAMGTYKYL	QKPMYEVQWK	DVEEINGNNY	VYIDPTQLPY	DHKWEFPRNR	LSFGKTLGAG	300
301	AFGKVVEATA	YGLIKSDAAM	TVAVKMLKPS	AHLTEREALM	SELKVLVSYLG	NHMNIANLLG	360
361	ACTIGGPTLV	ITEYCCYGDL	LNFLRRKRDS	FICSKQEDHA	EAALYKNLLH	SKESSCSDST	420
421	NEYMDMKPGV	SYVVPKADK	RRSVRIGSYI	ERDVTPAIME	DDELALDLED	LLSFSYQVAK	480
481	GMAFLASKNC	IHRDLAARNI	LLTHGRITKI	CDFGLARDIK	NDSNYVVKGN	ARLPVKWMAPE	540
541	ESIFNCVYTF	ESDVWSYGIF	LWELFSLGSS	PYPGMPVDSK	FYKMIKEGFR	MLSPEHAPAE	600
601	MYDIMKTCDW	ADPLKRPTFK	QIVQLIEKQI	SESTNHIYSN	LANCSPNRQK	PVVDHNSVRIN	660
661	SVGSTASSSQ	PLLVHDDV					720

1-218: GST Red: HIS6-tag Green: 3C clavage site blue: KIT fragment boxed: V559D&V654A mutation

KIT wt <sup>1</sup> Amino Acid Sequence							
1	MARGAWDF	LCVLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDETENENKQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKLFLV	120
121	DRSLYGKEDN	DTLVRCPPLTD	PEVTNYSKLG	CQGKPLPKDL	RFIPDPKAGI	MIKSVKRAYH	180
181	RLCLHCSVDQ	EGKSVLSEKF	ILKVRPAFKA	VPVSVSKAS	YLLREGEEFT	VTCTIKDVSS	240
241	SVYSTWKREN	SQTKLQEKYN	SWHHGDFNYE	RQATLTISSA	RVNDSGVFMC	YANNTFGSAN	300
301	VTTTLEVVDK	GFINIFPMIN	TTVFVNDGEN	VDLIVEYEAF	PKPEHQWIY	MNRTFTDKWE	360
361	DYPKSENESEN	IRYVSELHLT	RLKGTEGGTY	TFLVSNSDVN	AAIAFNYYVN	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	LKVLVSYLGNH	MNI <sup>V</sup> NLLGAC	660
661	TIGGPTLVIT	EYCCYGDLLN	FLRRKRDSFI	CSKQEDHAEA	ALYKNLLHSK	ESSCSDSTNE	720
721	YMDMKPGVSY	VVPTKADKRR	SVRIGSYIER	DVTPAIMEDD	ELALDLEDLL	SFSYQVAKGM	780
781	AFLASKNCIH	RDLAARNILL	THGRITKICD	FGLARDIKND	SNYVVKGNAR	LPVKWMAPE	840
841	IFNCVYTFES	DVWSYGIFLW	ELFSLGSSPY	PGMPVDSKPY	KMIKEGFRML	SPEHAPAEMY	900
901	DIMKTCDWAD	PLKRPTFKQI	VQLIEKQISE	STNHIYSNLA	NCSPNRQKPV	VDHNSVRINSV	960
961	GSTASSQPL	LHVHDDV					1020

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

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

Recombinant Proteins