

ProQinase™ KIT V559D/V654A

KIT proto-oncogene, receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCFR, c-Kit

Product No.: 1045-0000-1

Lot: 004

Description: Human KIT, C-terminal fragment, amino acids T₅₄₄-V₉₇₆ (as in [NCBI/Protein](#) entry NP_000213.1) with mutations V559D and V654A, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: KIT V559D/V654A Lot 004, was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 77,445 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 10 µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.096 µg/µl

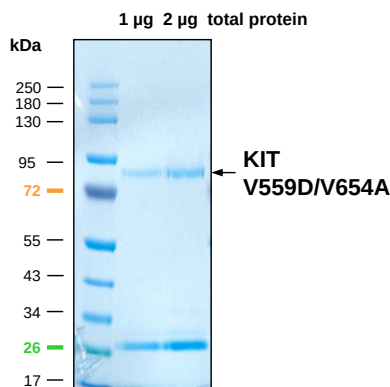
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

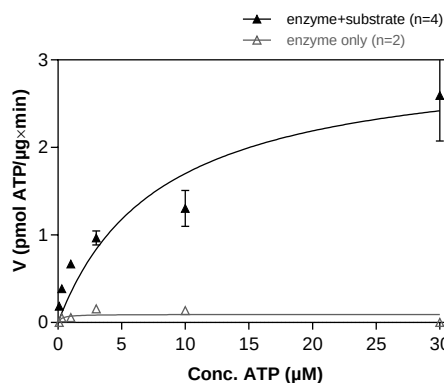
Specific kinase activity (P_i transfer): 3.1 pmol/µg x min

ATP-K_M: 8 µM

KIT V559D/V654A Lot 004:
Coomassie stain



KIT V559D/V654A Lot 004:
Determination of V_{max} and K_M value for ATP



- 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 10 µg/ml
Kinase: 4 µg/ml
- Filter binding assay
MSIP membrane (Millipore)

Sequence information

GST- KIT V559D/V654A Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RDSLEVLFGG	240
241	PLAMGTYKYL	QKPMYEVQWK	DVEEINGNNY	VYIDPTQLPY	DHKWEFPRNR	LSFGKTLGAG	300
301	AFGKVVEATA	YGLIKSDAAM	TVAVKMLKPS	AHLTEREALM	SELKVLSTYL	NHMNIANLLG	360
361	ACTIGGPITLV	ITEYCCYGDL	LNFLRRKRDS	FICSKQEDHA	EAALYKNLLH	SKESSCSDST	420
421	NEYMDMKPGV	SYVVPKADK	RRSVRIGSYI	ERDVTPAIME	DDELALDLED	LLSFSYQVAK	480
481	GMAFLASKNC	IHRDLAARNI	LLTHGRITKI	CDFGLARDIK	NDSNYVVKGN	ARLPVKWMAF	540
541	ESIFNCVYTF	ESDVWSYGIF	LWELFSLGSS	PYPGMPVDSK	FYKMIKEGFR	MLSPEHAPAE	600
601	MYDIMKTCWD	ADPLKRPTFK	QIVQLIEKQI	SESTNHIYSN	LANCSPNRQK	PVVDHVSVRIN	660
661	SVGSTASSSQ	PLLHVDDV					720

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: KIT fragment boxed: V559D, V654A

Kinase wt ¹ Amino Acid Sequence							
1	MARGARGAWDF	LCVLLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDETENENQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKLFLV	120
121	DRSLYGKEDN	DTLVRCPITD	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	PKPEHQQWIY	MNRTFTDKWE	360
361	DYPKSENESEN	IRYVSELHLT	RLKGTEGGTY	TFLVNSSDVN	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	LKVLSTYLGNH	MNIIVNLLGAC	660
661	TIGGPITLVIT	EYCCYGDLLN	FLRRKRDSFI	CSKQEDHAEA	ALYKNLLHSK	ESSCSDSTNE	720
721	YMDMKPGVSY	VVPTKADKRR	SVRIGSYIER	DVTPAIMEED	ELALDLEDLL	SFSYQVAKGM	780
781	AFLASKNCIH	RDLAARNILL	THGRITKICD	FGLARDIKND	SNYVVKGNAR	LPVKWMAFES	840
841	IFNCVYTFES	DVWSYGIFLW	ELFSLGSSPY	PGMPVDSKFY	KMIKEGFRML	SPEHAPAEMY	900
901	DIMKTCWDAD	PLKRPTFKQI	VQLIEKQISE	STNHIYSNLA	NCSPNRQKPV	VDHVSVRINSV	960
961	GSTASSSQPL	LHVDDV					1020

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

¹NCBI/Protein accession number NP_000213.1