

ProQinase™ KIT V654A

KIT proto-oncogene, receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCRF, c-Kit

Product No.: 0947-0000-1

Lot: 001

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

Product identity: KIT V654A Lot 001, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 77429 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: 0.077 µg/µl

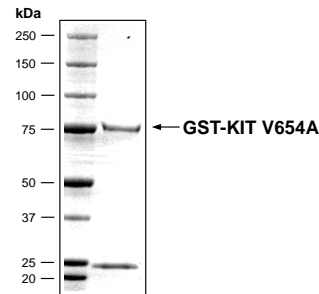
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 13 pmol/µg*min

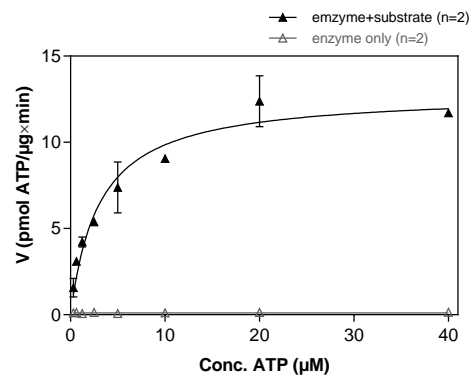
ATP-K_M: 3.1 µM

KIT V654A Lot001:
Coomassie stain



2.0 µg GST-KIT V654A

KIT V654A Lot001:
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: TRKC-derived peptide, 80 µg/ml
 - Kinase: 4 µg/ml

Assay technology:
Radiometric filter binding assay
MSPH membrane (96 well plate, Millipore)

Recombinant Proteins

Sequence information

GST-KIT V654A Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGQWQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVLFGG	240
241	PLAMGTYKYL	QKPMYEVQWK	VVEEINGNNY	VYIDPTQLPY	DHKWEFPRNR	LSFGKTLGAG	300
301	AFGKVVEATA	YGLIKSDAAM	TVAVKMLKPS	AHLTEREALM	SELKVLVSYL	NHMNIANLLG	360
361	ACTIGGPPLV	ITEYCCYDGL	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	MYDIMKTCWD	ADPLKRPTFK	QIVQLIEKQI	SESTNHIYSN	LANCSPNRQK	PVVDHSVRIN	660
661	SVGSTASSSQ	PLLVHDDV					720

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: KIT fragment **boxed**: variation from RefSeq

KIT wt ¹ Amino Acid Sequence							
1	MRGARGAWDF	LCVLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDETNNENQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKFLV	120
121	DRSLYGKEDN	DTLVRCPPLD	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	PMYEVQWKV	EEINGNNYVY	IDPTQLPYDH	KWEFPRNRLS	FGKTLGAGAF	600
600	GKVVEATAYG	LIKSDAAMTV	AVKMLKPSAH	LTEREALMSE	LKVLVSYLGNH	MNIVNLLGAC	660
661	TIGGPPLVIT	EYCCYDGLL	FLRRKRDSFI	CSKQEDHAEA	ALYKNLLHSK	ESSCSDSTNE	720
721	YMDMKPGVSY	VVPTKADKRR	SVRIGSYIER	DVTPAIMEED	ELALDLEDLL	SFSYQVAKGM	780
781	AFLASKNCIH	RDLAARNILL	THGRITKICD	FGLARDIKND	SNYVVKGNAR	LPVKWMAPE	840
841	IFNCVYTFES	DVWSYGIFLW	ELFSLGSSPY	PGMPVDSKPY	KMIKEGFRML	SPEHAPAEMY	900
901	DIMKTCWDAD	PLKRPTFKQI	VQLIEKQISE	STNHIYSNLA	NCSPNRQKPV	VDHSVRINSV	960
961	GSTASSSQPL	LHVHDDV					1020

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

¹[NCBI/Protein](https://www.ncbi.nlm.nih.gov/protein/000213.1) accession number NP_000213.1