

ProQinase™ KIT D816H

KIT proto-oncogene receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCFR, c-Kit

Product No.: 1041-0000-1

Lot: 005

Description: Human KIT, C-terminal fragment, amino acids T₅₄₄-V₉₇₆ (as in [NCBI/Protein](#) entry NP_000213.1) with a D816H mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: KIT D816H Lot 005 was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 77,479 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: 0.110 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

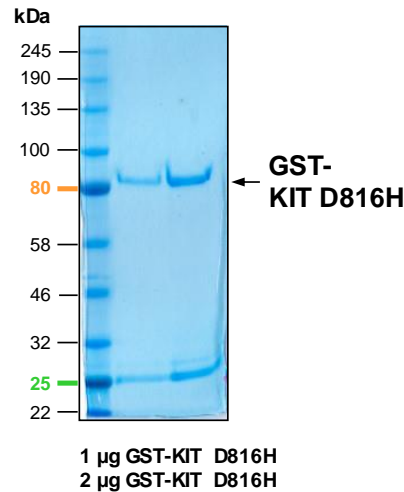
Specific kinase activity (P_i transfer): 48 pmol/µg × min
ATP-K_M: 3.2 µM

Additional assay technology:

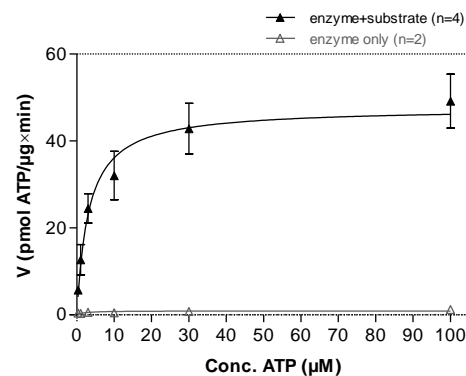
KIT D816H Lot 005 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

This product was manufactured at Reaction Biology in Freiburg, Germany, and is for in vitro research use only, not for use in humans or animals.
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KIT D816H Lot 005: Coomassie stain



KIT D816H Lot 005: Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:

- 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, 80 µg/ml
 - Kinase: 4 µg/ml
- Filter binding assay
- MSPH membrane (Millipore)

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GST-KIT D816H 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	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHG	RDS LEVLFQG	240
241	PLAMGTYKYL	QKPMYEVQWK	VVEEINGNNY	VYIDPTQLPY	DHKWEFPRNR	LSFGKTLGAG	300
301	AFGKVVEATA	YGLIKSDAAM	TVAVKMLKPS	AHLTEREALM	SELKVLISYLG	NHMNIVNLLG	360
361	ACTIGGPTLV	ITEYCCYGD	LNFLRRKRDS	FICSKQEDHA	EAALYKNLLH	SKESSCSDST	420
421	NEYMDMKPGV	SYVVPKADK	RRSVRIGSYI	ERDVTPAIME	DDELALDLED	LLSFSYQVAK	480
481	GMAFLASKNC	IHRDLAARNI	LLTHGRITKI	CDFGLARHIK	NDSNYVVKGN	ARLPVKWMAP	540
541	ESIFNCVYTF	ESDVWSYGIF	LWELFSLGSS	PYPGMPVDSK	FYKMIKEGFR	MLSPEHAPAE	600
601	MYDIMKTCWD	ADPLKRPTFK	QIVQLIEKQI	SESTNHIYSN	LANCSPNRQK	PVVDHVSVRIN	660
661	SVGSTASSQ	PLLVHDDV					720

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: KIT fragment **boxed**: D816H

KIT wt ¹ Amino Acid Sequence							
1	MRGARGAWDF	LCVLLLLLRV	QTGSSQPSVS	PGEPSPPSIH	PGKSDLIVRV	GDEIRLLCTD	60
61	PGFVKWTFEI	LDETENENQN	EWITEKAEAT	NTGKYTCTNK	HGLSNSIYVF	VRDPAKFLV	120
121	DRSLYGKEDN	DTLVRCPDLD	PEVTNYSKLG	CQKPLPKDL	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	TFLVSNSDVN	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	LKVLISYLGNH	MNIVNLLGAC	660
661	TIGGPTLVIT	EYCCYGDLLN	FLRRKRDSFI	CSKQEDHAEA	ALYKNLLHSH	ESSCSDSTNE	720
721	YMDMKPGVSY	VVPTKADKRR	SVRIGSYIER	DVTPAIMEED	ELALDLEDLL	SFSYQVAKGM	780
781	AFLASKNCIH	RDLAARNILL	THGRITKICD	FGLARDIKND	SNYVVKGNAR	LPVKWMAPEP	840
841	IFNCVYTFES	DVWSYGIFLW	ELFSLGSSPY	PGMPVDSKPY	KMIKEGFRML	SPEHAPAEMY	900
901	DIMKTCWDAD	PLKRPTFKQI	VQLIEKQISE	STNHIYSNLA	NCSPNRQKPV	VDHVSVRINSV	960
961	GSTASSQPL	LHVHDDV					1020

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

¹[NCBI/Protein](#) accession number NP_000213.1