Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ KIT D816V

KIT proto-oncogene receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: KIT

Synonyms: CD117, PBT, SCFR, c-Kit

Product No.: 0946-0000-1

Lot: 001

Description: Human KIT, C-terminal fragment, amino acids T_{544}-V_{976} (as in NCBI/Protein entry NP_000213.1) with a D816V mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: KIT D816V Lot 002 was confirmed as KIT by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 77,441 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

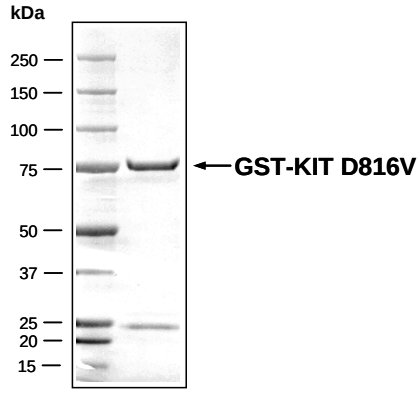
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

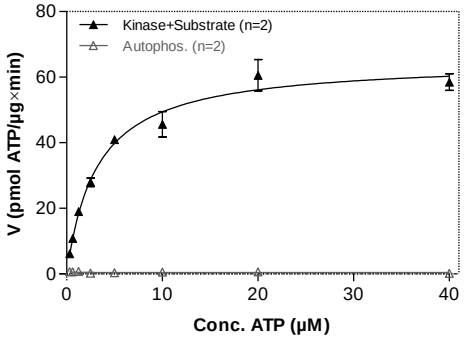
Biochemical Parameters:
Specific kinase activity (P_i transfer): 65 pmol/µg × min
ATP-K_M: 3.2 µM

KIT D816V Lot 001:
Coomassie stain



2.0 µg GST-KIT D816V

KIT D816V Lot 001:
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 $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg / ml $PEG_{20.000}$
  ATP (variable)
  Substrate: TRK-C-derived peptide, 80 µg/ml
  KIT D816V: 4 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
KIT D816V Lot 001 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

ProQinase™ KIT D816V

Product No.: 0946-0000-1

GST-KIT D816V Recombinant Fusion Protein Amino Acid Sequence

```
1    MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
61   GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV  120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK  180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHG RDSLEVLFQG  240
241  PLAMGTYKYL QKPMYEVQWK VVEEINGNNY VYIDPTQLPY DHKWEFPRNR LSFGKTLGAG  300
301  AFGKVVEATA YGLIKSDAAM TVAVKMLKPS AHLTEREALM SELKVLSYLG NHMNIVNLLG  360
361  ACTIGGPTLV ITEYCCYGDL LNFLRRKRDS FICSKQEDHA EAALYKNLLH SKESSCSDST  420
421  NEYMDMKPGV SYVVPTKADK RRSVRIGSYI ERDVTPAIME DDELALDLED LLSFSYQVAK  480
481  GMAFLASKNC IHRDLAARNI LLTHGRITKI CDFGLARVIK NDSNYVVKGN ARLPVKWMAP  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 LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV  120
 121  DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH  180
 181  RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS  240
 241  SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN  300
 301  VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE  360
 361  DYPKSENESN 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 LKVLSYLGNH MNIVNLLGAC  660
 661  TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE  720
 721  YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM  780
 781  AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES  840
 841  IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY  900
 901  DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV  960
 961  GSTASSSQPL LVHDDV                                                 1020
```

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

[1]NCBI/Protein accession number NP_000213.1

Recombinant Proteins