

Certificate of Analysis

FLT3 wt

fms-related tyrosine kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: FLT3

Synonyms: CD135, FLK2, STK-1

Product No.: 0776-0000-1

Lot: 011

Description: Human FLT3, C-terminal fragment, amino acids R₅₇₁-S₉₉₃ (as in NCBI/Protein entry NP_004110.2), N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: FLT3 wt Lot 011 was confirmed as FLT3 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 77,198 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

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: 0.109 µg/µl

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

Biochemical Parameters:

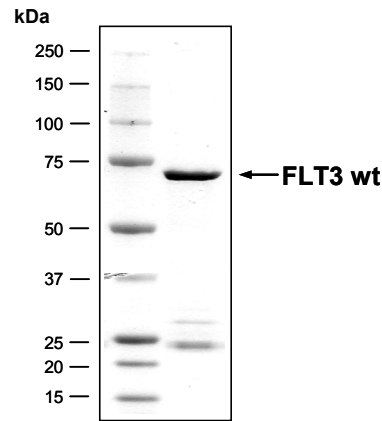
Specific kinase activity (P_i transfer): 212 pmol/µg×min

ATP-K_M: 8.3 µM

Additional assay technology: FLT3 wt Lot 011

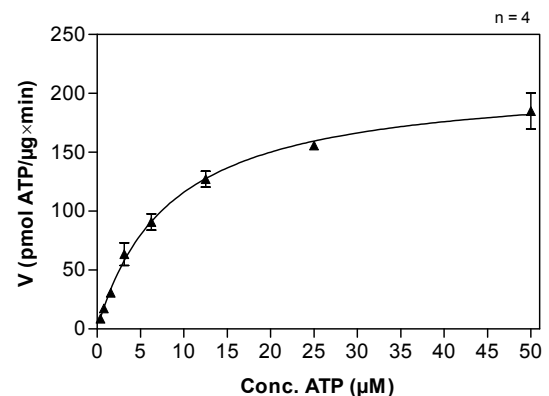
was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from **Promega**
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

**FLT3 wt Lot 011:
Coomassie stain**



2.0 µg GST-FLT3 wt

**FLT3 wt Lot 011:
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: Poly(Ala,Glu,Lys,Tyr)_{6:2.5:1}, 20 µg/ml
 - FLT3 wt: 1 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

FLT3 wt

Product No.: 0776-0000-1

| FLT3 wt 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 | KRIEAIPOID | KYLKSSKYIA | WPLQGWQATF | GGGDHPPKSD | PMGHHHHHG | RDSLEVLFOG | 240 |
| 241 | PLAMGRYESQ | LQMVQVTGSS | DNEYFYVDFR | EYEYDLKWEF | PRENLEFGKV | LGSGAFGKVM | 300 |
| 301 | NATAYGISKT | GVSIVAVKVM | LKEKADSSER | EALMSELKMM | TQLGSHENIV | NLLGACTLSG | 360 |
| 361 | PIYLIFEYCC | YGDLLNLYRS | KREKFHRTWT | EIFKEHNFSF | YPTFQSH PNS | SMPGSREVQI | 420 |
| 421 | HPDSDQISGL | HGNSFHSEDE | IEYENQKRL | EEEDLVLTTF | EDLLCFAYQV | AKGMEFLEFK | 480 |
| 481 | SCVHRDLAAR | NVLVTHGKVV | KICDFGLARD | IMSDSNVYVR | GNARLPVKWM | APESLFEGIY | 540 |
| 541 | TIKSDVWSYG | ILLWEIFSLG | VNPYPGIPVD | ANFYKLIQNG | FKMDQPFYAT | EEIYIIMQSC | 600 |
| 600 | WAFDSRKRPS | FPNLTSEFLG | QLADAEEMY | QNV DGRVSEC | PHTYQNRPPF | SREMDLGLLS | 660 |
| 661 | PQAQVEDS | | | | | | 720 |

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue:** FLT3 wt fragment

| FLT3 wt wt ¹ Amino Acid Sequence | | | | | | | |
|---|--------------------|--------------------|--------------------|--------------------|-------------------|--------------------|-----|
| 1 | MPALARDGGQ | LPLLVVFSAM | IFGTITNQDL | PVIKCVLINH | KNNDSSVGKS | SSYPMVSESP | 60 |
| 61 | EDLGCALRPQ | SSGTVYEA AAA | VEVDVSASIT | LQVLVDAPGN | ISCLWVFKHS | SLNCQPHFDL | 120 |
| 121 | QNRGVVSMVI | LKMTETQAGE | YLLFIQSEAT | NYTILFTVSI | RNTLLYTLRR | PYFRKMENQD | 180 |
| 181 | ALVCISESVP | EPIVEWVLCD | SQGESCKEES | PAVVKKEEKV | LHELFGTDIR | CCARNELGRE | 240 |
| 241 | CTRLFTIDLN | QTPQTTL PQL | FLKVGEPLWI | RCKAVHVNHG | FGLTWELENK | ALEEGNYFEM | 300 |
| 301 | STYSTNRMTI | RILFAFVSSV | ARNDTGY YTC | SSSKHPSQSA | LVTIVEKGF I | NATNSSEDEY | 360 |
| 361 | IDQYEEFCFS | VRFKAYPQIR | CTWTF SRKSF | PCEQKGLDNG | YSISKFCN HK | HQPGEYIFHA | 420 |
| 421 | ENDDAQFTKM | FTLNIRRPQ | VLAEASASQA | SCFSDGYPLP | SWTWK KCDK | SPNCTEEITE | 480 |
| 481 | GVWNRKANRK | VFGQWVSSST | LN MSEAIKGF | LVKCCAYNSL | GTSCETILLN | SPGPFPIQD | 540 |
| 541 | NISFYATIGV | CLLFIVVLT L | LICHKYKKQF | RYESQLQMVQ | VTGSSDNEYF | YVDFREYEYD | 600 |
| 600 | LKWEFPRENL | EFGKVLGSGA | FGKVMNATAY | GISKTGVS IQ | VAVKMLKEKA | DSSEREALMS | 660 |
| 661 | ELKMMTQLGS | HENIVNLLGA | CTLSGPIYLI | FEYCCYGDLL | NYLRSKREKF | HRTWTEIFKE | 720 |
| 721 | HNF SFYPTFQ | SHPNSSMPGS | REVQIHPDS D | QISGLHGNSF | HSEDEIEYEN | QKRLEEEEDL | 780 |
| 781 | NVLTFEDLLC | FAYQVAKGME | FLEFKSCVHR | DLAARNVLT | HGKVVKICDF | GLARDIMSDS | 840 |
| 841 | NYVVRGNARL | PVKWMAPESL | FEGIYTIKSD | VWSYGILLWE | IFSLGVNPYP | GIPVDANFYK | 900 |
| 901 | LIQNGFKMDQ | PFYATEE IYI | IMQSCWAFDS | RKRPSFPNLT | SFLGCQLADA | EEAMYQNV DQ | 960 |
| 961 | RVSECPHTYQ | NRRPFSREMD | LGLLSPQAQV | EDS | | | |

blue: FLT3 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_004110.2