

## FLT3 D835Y

fms related tyrosine kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: FLT3

Synonyms: CD135, EC 2.7.10.1, FLK2, STK1

Product No.: 0777-0000-1

Lot: 009

**Description:** Human FLT3, C-terminal fragment, amino acids R<sub>571</sub>-S<sub>993</sub> (as in [NCBI/Protein](#) entry NP\_004110.2), D835Y mutation, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** FLT3 D835Y Lot 009, was confirmed as FLT3 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 77,246 Da

**Expression host:** Sf9 insect 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:** 0.245 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

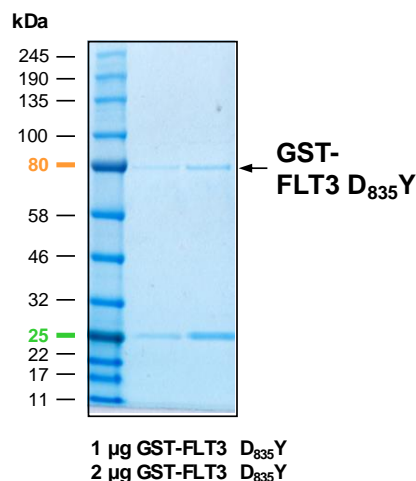
### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 27 pmol/µg × min  
ATP-K<sub>M</sub>: 2.4 µM

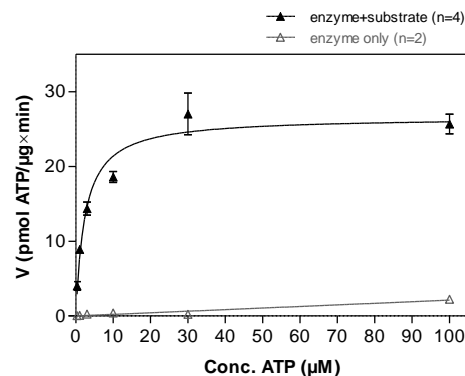
### Additional assay technology:

FLT3 D835Y Lot 009 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 D835Y Lot 009: Coomassie stain



### FLT3 D835Y Lot 009: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: TRK-C derived peptide 80 µg/ml
  - Kinase: 2 µg/ml
- Filter binding assay
  - MSPH membrane (Millipore)

## FLT3 D835Y

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GST-FLT3 D835Y Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPE	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHG</b>	RDS <b>LEVLFGQ</b>	240
241	<b>PLAMGRYESQ</b>	<b>LQMVQVTGSS</b>	<b>DNEYFYVDFR</b>	<b>EYEYDLKWEF</b>	<b>PRENLEFGKV</b>	<b>LGSGAFGKVM</b>	300
301	<b>NATAYGISKT</b>	<b>GVSIVAVKM</b>	<b>LKEKADSSER</b>	<b>EALMSELKMM</b>	<b>TQLGSHENIV</b>	<b>NLLGACTLSG</b>	360
361	<b>PIYLIFEYCC</b>	<b>YGDLLNYLRS</b>	<b>KREKFHRTWT</b>	<b>EIFKEHNFSF</b>	<b>YPTFQSH PNS</b>	<b>SMPGSREVQI</b>	420
421	<b>HPDSDQISGL</b>	<b>HGNSFHSEDE</b>	<b>IEYENQKRLE</b>	<b>EEEDLNVLTF</b>	<b>EDLLCFAYQV</b>	<b>AKGMEFLEFK</b>	480
481	<b>SCVHRDLAAR</b>	<b>NVLVTHGKVV</b>	<b>KICDFGLARY</b>	<b>IMSDSNYVVR</b>	<b>GNARLPVKWM</b>	<b>APESLFEGIY</b>	540
541	<b>TIKSDVWSYG</b>	<b>ILLWEIFSLG</b>	<b>VNPYPGIPVD</b>	<b>ANFYKLIQNG</b>	<b>FKMDQPFYAT</b>	<b>EIYIIMQSC</b>	600
601	<b>WAFDSRKRPS</b>	<b>FPNLTSFLGC</b>	<b>QLADAEAMY</b>	<b>QNVDRVSEC</b>	<b>PHTYQNRPF</b>	<b>SREMDLGLS</b>	660
661	<b>PQAQVEDS</b>						720

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: FLT3 fragment **boxed**: D835Y mutation

FLT3 wt <sup>1</sup> Amino Acid Sequence							
1	MPALARDGGQ	LPLLVVFSAM	IFGTITNQDL	PVIKCVLINH	KNNDSSVGKS	SSYPMVSESP	60
61	EDLGCALRPQ	SSGTVYEAAA	VEVDVSASIT	LQVLVDAPGN	ISCLWVFKHS	SLNCQPHFDL	120
121	QNRGVVSMVI	LKMTETQAGE	YLLFIQSEAT	NYTILFTVSI	RNTLLYTLRR	PYFRKMNQD	180
181	ALVCISESVP	EPIVEWVLCD	SQGESCKEES	PAVVKKEEKV	LHELFGTDIR	CCARNELGRE	240
241	CTRLFTIDLN	QTPQTTLPL	FLKVGPELWI	RCKAVHVNHG	FGLTWELENK	ALEEGNYFEM	300
301	STYSTNRMTI	RILFAFVSSV	ARNDTGYTTC	SSSKHPSQSA	LVTIVEKGF I	NATNSSEDEY	360
361	IDQYEEFCFS	VRFKAYPQIR	CTWTFSRKSF	PCEQKGLDNG	YSISKFCNHNK	HQPGEYIFHA	420
421	ENDDAQFTKM	FTLNIRRKPK	VLAEASASQA	SCFSDGYPLP	SWTWKCKSDK	SPNCTEEITE	480
481	GVWNRKANRK	VFGQWVSSST	LMNSEAIKGF	LVKCCAYNSL	GTSCETILLN	SPGPFPIQD	540
541	NISFYATIGV	CLLFIVVLT	LICHKYKKQF	<b>RYESQLQMVQ</b>	<b>VTGSSDNEYF</b>	<b>YVDFREYEYD</b>	600
601	<b>LKWEFPRENL</b>	<b>EFGKVLGSGA</b>	<b>FGKVMNATAY</b>	<b>GISKTGVS IQ</b>	<b>VAVKMLKEKA</b>	<b>DSSEREALMS</b>	660
661	<b>ELKMMTQLGS</b>	<b>HENIVNLLGA</b>	<b>CTLSGPIYLI</b>	<b>FEYCCYGDLL</b>	<b>NYLRSKREKF</b>	<b>HRTWTEIFKE</b>	720
721	<b>HNFSFYPTFQ</b>	<b>SHPNSSMPGS</b>	<b>REVQIHPDS</b>	<b>QISGLHGNSF</b>	<b>HSEDEIEYEN</b>	<b>QKRLEEEEDL</b>	780
781	<b>NVLTFFEDLLC</b>	<b>FAYQVAKGME</b>	<b>FLEFKSCVHR</b>	<b>DLAARNVLVT</b>	<b>HGKVVKICDF</b>	<b>GLARDIMSDS</b>	840
841	<b>NYVVRGNARL</b>	<b>PVKWMAPESE</b>	<b>FEGIYTIKSD</b>	<b>VWSYGILLWE</b>	<b>IFSLGVNYPY</b>	<b>GIPVDANFYK</b>	900
901	<b>LIQNGFKMDQ</b>	<b>PFYATEEIIYI</b>	<b>IMQSCWAFDS</b>	<b>RKRPSFPNLT</b>	<b>SFLGCQLADA</b>	<b>EEAMYQNVGD</b>	960
961	<b>RVSECPHTYQ</b>	<b>NRRPFSREMD</b>	<b>LGLLSPQAQV</b>	<b>EDS</b>			1020

**blue**: FLT3 sequence expressed in recombinant protein **Red**: variant in recombinant protein

<sup>1</sup>[NCBI/Protein](#) accession number NP\_004110.2

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