

ProQinase™ FLT3 ITD

fms related tyrosine kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: FLT3

Synonyms: CD135, EC 2.7.10.1, FLK2, STK1

Product No.: 0778-0000-1

Lot: 007

Description: Human FLT3, C-terminal fragment, amino acids R₅₇₁-S₉₉₃ (as in [NCBI/Protein](#) entry NP_004110.2), internal tandem duplication (oncological mutation: ITD) of aa 591-600, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: FLT3 ITD Lot 007, was confirmed as FLT3 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 78,552 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: 0.088 µg/µl

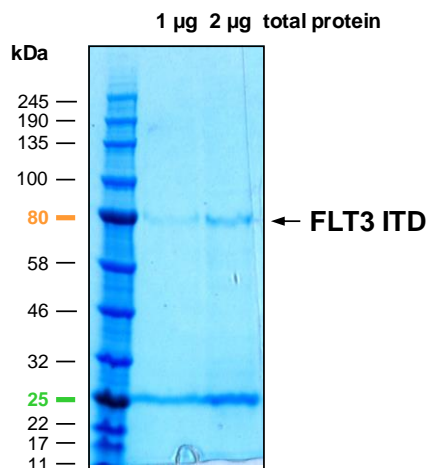
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

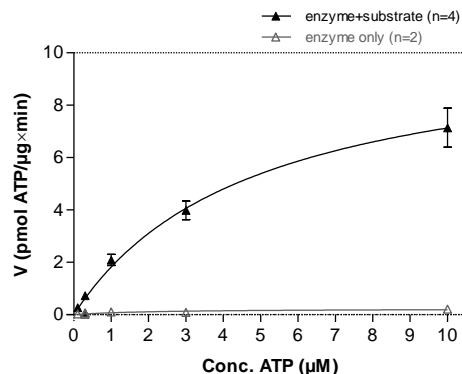
Specific kinase activity (P_i transfer): 11 pmol/µg × min

ATP-K_M: 4.8 µM

FLT3 ITD Lot 007: Coomassie stain



FLT3 ITD Lot 007: 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 10 µg/ml
 - Kinase: 1 µg/ml
- Filter binding assay
MSIP membrane (Millipore)

Additional assay technology:

FLT3 ITD Lot 007 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

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

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

FLT3 wt ¹ 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	LVTIVEKGFI	NATNSSEDEY	360
361	IDQYEEFCFS	VRFKAYPQIR	CTWTFSRKSF	PCEQKGLDNG	YSISKFCNHN	HQPGEYIFHA	420
421	ENDDAQFTKM	FTLNIRRPQ	VLAEASASQA	SCFSDGYPLP	SWTWKCKSDK	SPNCTEEITE	480
481	GVWNRKANRK	VFGQWVSSST	LNMSIAIKGF	LVKCCAYNSL	GTSCETILLN	SPGPFPPFIQD	540
541	NISFYATIGV	CLLFIVVLT	LICHKYKKQF	RYESQLQMVQ	VTGSSDNEYF	YVDFREYEYD	600
601	LKWEFPREN	EFKVLGSGA	FGKVMNATAY	GISKTGVSQ	VAVKMLKEKA	DSSEREALMS	660
661	ELKMMTQLGS	HENIVNLLGA	CTLSGPIYLI	FEYCCYGDLL	NYLRSKREKF	HRTWTEIFKE	720
721	HNFSFYPTFQ	SHPNSSMPGS	REVQIHPDSD	QISGLHGNSF	HSEDEIEYEN	QKRLEEEEDL	780
781	NVLTFFEDLLC	FAYQVAKGME	FLEFKSCVHR	DLAARNVLVT	HGKVVKICDF	GLARDIMSDS	840
841	NYVVRGNARL	PVKWMAPESL	FEGIYTIKSD	VWSYGILLWE	IFSLGVNYP	GIPVDANFYK	900
901	LIQNGFKMDQ	PFYATEEIIYI	IMQSCWAFDS	RKRPSFPNLT	SFLGCQLADA	EEAMYQNVGD	960
961	RVSECPHTYQ	NRRPFSREMD	LGLLSPQAQV	EDS			1020

blue: FLT3 sequence expressed in recombinant protein **Red**: duplicated sequence in FLT3 ITD

¹[NCBI/Protein](#) accession number NP_004110.2