MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of active FLT4 [800 - 1297]

Enzyme description:- FLT4 [800 - 1297]

Clone number:- DU 68244

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	82, 625.44 daltons
Average Mass	82, 678.72 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.99

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT,

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
GGEEEEYFELVKKK Final concentration: 300 uM

Clone Data Sheet

FLT4 [800 - 1297]

Protein	FLT4 [800 - 1297]
Clone number	DU 68244
Species	Human
Accession number	NM_182925.5
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGPLGS**MRRPAHADIKTGYLSIIMDPGEVPLEEQCEYLS** **YDASQWEFPRERLHLGRVLGYGAFGKVVEASAFGIHKGSSCDTV** **AVKMLKEGATASEHRALMSELKILIHIGNHLNVVNLLGACTKPQ** **GPLMVIVEFCKYGNLSNFLRAKRDAFSPCAEKSPEQRGRFRAMV** **ELARLDRRRPGSSDRVLFARFSKTEGGARRASPDQEAEDLWLSP** **LTMEDLVCYSFQVARGMEFLASRKCIHRDLAARNILLSESDVVK** **ICDFGLARDIYKDPDYVRKGSARLPLKWMAPESIFDKVYTTQSD** **VWSFGVLLWEIFSLGASPYPGVQINEEFCQRLRDGTRMRAPELA** **TPAIRRIMLNCWSGDPKARPAFSELVEILGDLLQGRGLQEEEEV** **CMAPRSSQSSEEGSFSQVSTMALHIAQADAEDSPPSLQRHSLAA** **RYYNWVSFPGCLARGAETRGSSRMKTFEEFPMTPTTYKGSVDNQ** **TDSGMVLASEEFEQIESRHRQESGF**
Native sequence	Amino acids M800 – F1297 (end residue is Y1363) of human FLT4. Residue M232 of the fusion protein is equivalent to M800 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1sites of pFastBac Dual.

Nucleotide sequence of insert

ggatccATGAGGAGGCCGGCCCACGCAGACATCAAGACGGGCTAC
CTGTCCATCATCATGGACCCCGGGGAGGTGCCTCTGGAGGAGCAA
TGCGAATACCTGTCCTACGATGCCAGCCAGTGGGAATTCCCCCGA
GAGCGGCTGCACCTGGGGAGAGTGCTCGGCTACGGCGCCTTCGGG
AAGGTGGTGGAAGCCTCCGCTTTCGGCATCCACAAGGGCAGCAGC
TGTGACACCGTGGCCGTGAAAATGCTGAAAGAGGGCGCCACGGCC
AGCGAGCACCGCGCGCTGATGTCGGAGCTCAAGATCCTCATTCAC
ATCGGCAACCACCTCAACGTGGTCAACCTCCTCGGGGCGTGCACC
AAGCCGCAGGGCCCCCTCATGGTGATCGTGGAGTTCTGCAAGTAC
GGCAACCTCTCCAACTTCCTGCGCGCCAAGCGGGACGCCTTCAGC
CCCTGCGCGGAGAAGTCTCCCGAGCAGCGCGGACGCTTCCGCGCC
ATGGTGGAGCTCGCCAGGCTGGATCGGAGGCGGCCGGGGAGCAGC
GACAGGGTCCTCTTCGCGCGGTTCTCGAAGACCGAGGGCGGAGCG
AGGCGGGCTTCTCCAGACCAAGAAGCTGAGGACCTGTGGCTGAGC
CCGCTGACCATGGAAGATCTTGTCTGCTACAGCTTCCAGGTGGCC
AGAGGGATGGAGTTCCTGGCTTCCCGAAAGTGCATCCACAGAGAC
CTGGCTGCTCGGAACATTCTGCTGTCGGAAAGCGACGTGGTGAAG
ATCTGTGACTTTGGCCTTGCCCGGGACATCTACAAAGACCCCGAC
TACGTCCGCAAGGGCAGTGCCCGGCTGCCCCTGAAGTGGATGGCC
CCTGAAAGCATCTTCGACAAGGTGTACACCACGCAGAGTGACGTG
TGGTCCTTTGGGGTGCTTCTCTGGGAGATCTTCTCTCTGGGGGCC
TCCCCGTACCCTGGGGTGCAGATCAATGAGGAGTTCTGCCAGCGG
CTGAGAGACGGCACAAGGATGAGGGCCCCGGAGCTGGCCACTCCC
GCCATACGCCGCATCATGCTGAACTGCTGGTCCGGAGACCCCAAG
GCGAGACCTGCATTCTCGGAGCTGGTGGAGATCCTGGGGGACCTG
CTCCAGGGCAGGGGCCTGCAAGAGGAAGAGGAGGTCTGCATGGCC
CCGCGCAGCTCTCAGAGCTCAGAAGAGGGCAGCTTCTCGCAGGTG
TCCACCATGGCCCTACACATCGCCCAGGCTGACGCTGAGGACAGC
CCGCCAAGCCTGCAGCGCCACAGCCTGGCCGCCAGGTATTACAAC
TGGGTGTCCTTTCCCGGGTGCCTGGCCAGAGGGGCTGAGACCCGT
GGTTCCTCCAGGATGAAGACATTTGAGGAATTCCCCATGACCCCA
ACGACCTACAAAGGCTCTGTGGACAACCAGACAGACAGTGGGATG
GTGCTGGCCTCGGAGGAGTTTGAGCAGATAGAGAGCAGGCATAGA
CAAGAAAGCGGCTTCtgagcggccgc