MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active FGFR3 [436 - 806]

Enzyme description:- FGFR3 [436 – 806]

Clone number:- DU 67062

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 67, 934.34 daltons
Average Mass 67, 978.23 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.58

Purity:- >75 %

Activation protocol:- Constitutively Active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 10 mM DTT, 10 mM magnesium acetate, 0. 1mM EGTA

Substrate:-
Poly Glu:Tyr (4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

FGFR3 [436 – 806]

Protein	FGFR3 [436 – 806]
Clone number	DU 67062
Species	Human
Accession number	NM_000142.4
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PLVRIARLS** **SGEGPTLANVSELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAI** **GIDKDRAAKPVTVAVKMLKDDATDKDLSDLVSEMEMMKMIGKHKNIIN** **LLGACTQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQ** **LTFKDLVSCAYQVARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADF** **GLARDVHNLDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLL** **WEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWH** **AAPSQRPTFKQLVEDLDRVLTVTSTDEYLDLSAPFEQYSPGGQDTPSS** **SSSGDDSVFAHDLLPPAPPSSGGSRT**
Native sequence	Amino acids P436 – T806 (end) of human FGFR3. Residue P232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 - *Not*1 sites of pFastBac GST

Nucleotide sequence of insert

ggatcccCACTGGTGCGCATCGCAAGGCTGTCCTCAGGGGAGGGCCCC
ACGCTGGCCAATGTCTCCGAGCTCGAGCTGCCTGCCGACCCCAAATGG
GAGCTGTCTCGGGCCCGGCTGACCCTGGGCAAGCCCCTTGGGGAGGGC
TGCTTCGGCCAGGTGGTCATGGCGGAGGCCATCGGCATTGACAAGGAC
CGGGCCGCCAAGCCTGTCACCGTAGCCGTGAAGATGCTGAAAGACGAT
GCCACTGACAAGGACCTGTCGGACCTGGTGTCTGAGATGGAGATGATG
AAGATGATCGGGAAACACAAAAACATCATCAACCTGCTGGGCGCCTGC
ACGCAGGGCGGGCCCCTGTACGTGCTGGTGGAGTACGCGGCCAAGGGT
AACCTGCGGGAGTTTCTGCGGGCGCGGCGGCCCCCGGGCCTGGACTAC
TCCTTCGACACCTGCAAGCCGCCCGAGGAGCAGCTCACCTTCAAGGAC
CTGGTGTCCTGTGCCTACCAGGTGGCCCGGGGCATGGAGTACTTGGCC
TCCCAGAAGTGCATCCACAGGGACCTGGCTGCCCGCAATGTGCTGGTG
ACCGAGGACAACGTGATGAAGATCGCAGACTTCGGGCTGGCCCGGGAC
GTGCACAACCTCGACTACTACAAGAAGACAACCAACGGCCGGCTGCCC
GTGAAGTGGATGGCGCCTGAGGCCTTGTTTGACCGAGTCTACACTCAC
CAGAGTGACGTCTGGTCCTTTGGGGTCCTGCTCTGGGAGATCTTCACG
CTGGGGGGCTCCCCGTACCCCGGCATCCCTGTGGAGGAGCTCTTCAAG
CTGCTGAAGGAGGGCCACCGCATGGACAAGCCCGCCAACTGCACACAC
GACCTGTACATGATCATGCGGGAGTGCTGGCATGCCGCGCCCTCCCAG
AGGCCCACCTTCAAGCAGCTGGTGGAGGACCTGGACCGTGTCCTTACC
GTGACGTCCACCGACGAGTACCTGGACCTGTCGGCGCCTTTCGAGCAG
TACTCCCCGGGTGGCCAGGACACCCCCAGCTCCAGCTCCTCAGGGGAC
GACTCCGTGTTTGCCCACGACCTGCTGCCCCCGGCCCCACCCAGCAGT
GGGGGCTCGCGGACGtgagcggccgc