

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Fibroblast Growth Factor Receptor 1 [400 - 820]

<u>Enzyme description:-</u>	FGF-R1 [400 - 820]
<u>Clone number:-</u>	DU 4435
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	5 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	50, 654.23 daltons
Average Mass	50, 686.88 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.42
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
Poly Glu Tyr (4:1)	Final concentration: 1 mg/ml
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

Fibroblast Growth Factor Receptor 1 [400 - 820]

<u>Protein</u>	FGF-R1 [400 – 820]
<u>Clone number</u>	DU 4435
<u>Species</u>	Human
<u>Accession number</u>	M34641
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMGSGTKKSDFHSMQMAVHKLAKSIP LRRQVTVSADSSASMNSGVLLVRPSRLSSSGTPMLAGVSEYELPEDPRW ELPRDRLVLGKPLGEGCFGQVVLAEAI GLDKDKPNRVTKVAVKMLKSDA TEKDLSDLISEMEMMKMIGKHKNI INLLGACTQDGPLYVIVEYASKGNL REYLQARRPPGLEYCYNPSHNPEEQ LSSKDLVSCAYQVARGMEYLASKK CIHRDLAARNVLVTEDNVMKIADFG LARDIHHIDYYKKT TNGRLPVKWM APEALFDRIYTHQSDVWSFGVLLWEI FT LGGSPYPGVPVEELFKLLKEG HRMDKPSNCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRIVALTSNQE YLDLSMPLDQYSPSPDTRSSTCSSGEDSVFSHEPLPEEPCLPRHPAQL ANGGLKRR
<u>Native sequence</u>	Amino acids 6400 – R820 (end) of human FGF-R1. Residue G29 of the fusion protein is equivalent to G400 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pFastBAC HTb

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Complete nucleotide Sequence

atgtcgtactaccatcaccatcaccatcacgattacgatatcccaacga
ccgaaaacctgtattttcagggcgccatgggatccGGTACCAAGAAGAG
TGACTTCCACAGCCAGATGGCTGTGCACAAGCTGGCCAAGAGCATCCCT
CTGCGCAGACAGGTAACAGTGTCTGCTGACTCCAGTGCATCCATGAACT
CTGGGGTTCTTCTGGTTCGGCCATCACGGCTCTCCTCCAGTGGGACTCC
CATGCTAGCAGGGGTCTCTGAGTATGAGCTTCCCGAAGACCCTCGCTGG
GAGCTGCCTCGGGACAGACTGGTCTTAGGCCAAACCCCTGGGAGAGGGCT
GCTTTGGGCAGGTGGTGTGGCAGAGGCTATCGGGCTGGACAAGGACAA
ACCCAACCGTGTGACCAAAGTGGCTGTGAAGATGTTGAAGTCGGACGCA
ACAGAGAAAGACTTGTGAGACCTGATCTCAGAAATGGAGATGATGAAGA
TGATCGGGAAGCATAAGAATATCATCAACCTGCTGGGGGCCTGCACGCA
GGATGGTCCCTTGTATGTCATCGTGGAGTATGCCTCCAAGGGCAACCTG
CGGGAGTACCTGCAGGCCCCGAGGCCCCCAGGGCTGGAATACTGCTACA
ACCCAGCCACAACCCAGAGGAGCAGCTCTCCTCCAAGGACCTGGTGTGTC
CTGCGCCTACCAGGTGGCCCCGAGGCATGGAGTATCTGGCCTCCAAGAAG
TGCATACACCGAGACCTGGCAGCCAGGAATGTCCTGGTGACAGAGGACA
ATGTGATGAAGATAGCAGACTTTGGCCTCGCACGGGACATTACCCACAT
CGACTACTATAAAAAGACAACCAACGGCCGACTGCCTGTGAAGTGGATG
GCACCCGAGGCATTATTTGACCGGATCTACACCCACCAGAGTGATGTGT
GGTCTTTCGGGGTGCTCCTGTGGGAGATCTTCACTCTGGGCGGCTCCCC
ATACCCCGGTGTGCCTGTGGAGGAACTTTCAAGCTGCTGAAGGAGGGT
CACCGCATGGACAAGCCCAGTAACTGCACCAACGAGCTGTACATGATGA
TGCGGGACTGCTGGCATGCAGTGCCCTCACAGAGACCCACCTTCAAGCA
GCTGGTGGAAGACCTGGACCGCATCGTGGCCTTGACCTCCAACCAGGAG
TACCTGGACCTGTCCATGCCCCCTGGACCAGTACTCCCCCAGCTTTCCCG
ACACCCGGAGCTCTACGTGCTCCTCAGGGGAGGATTCCGTCTTCTCTCA
TGAGCCGCTGCCCAGGAGCCCTGCCTGCCCCGACACCCAGCCAGCTT
GCCAATGGCGGACTCAAACGCCGctgagcggccgc