

University of Dundee

Standard Operating Procedure

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

Enzyme description:- FGF-R1 [400 - 820]

Clone number:- DU 4434

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni²⁺-NTA agarose

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 50, 654.23 daltons
Average Mass 50, 686.88 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.42

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, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

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

Specific activity range:- To be determined

Clone Data Sheet

Fibroblast Growth Factor Receptor 1 [400 - 820]

Protein FGF-R1 [400 – 820]

Clone number DU 4435

Species Human

Accession number M34641

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGSGTKKSDFHSMQMAVHKLAKSIP
LRRQVTVSADSSASMNSGVLLVRPSRLSSSGTPMLAGVSEYELPEDPRW
ELPRDRLVLGKPLGEGCFQVVLAEAIGLDKDKPNRVTKVAVKMLKSDA
TEKDLSDLISEMEMMKMIGKHKNINLLGACTQDGPLYVIVEYASKGNL
REYLQARRPPGLECYNPSHNPEEQLSKDLVSCAYQVARGMEYLASKK
CIHRDLAARNVLVTEDNVMKIADFGIARDIHHIDYYKKTNGRLPVKWM
APEALFDRIYTHQSDVWSFGVLLWEIFTLGGSPYPGPVVEELFKLLKEG
HRMDKPSNCTNELYMMRDCWHAVPSQRPTFKQLVEDLDRIVALTSNQE
YLDLSMPLDQYSPSPDTRSSTCSSGEDSVFSHEPLPEEPCLPRHPAQL
ANGGLKRR

Native sequence Amino acids G400 – 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.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1 and *Not*1 sites of pFastBAC HTb

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**Complete
nucleotide
Sequence**

atgtcgtactaccatcaccatcaccatcacgattacgatatcccaacga
ccgaaaacctgtatthttcagggcgccatgggatccGGTACCAAGAAGAG
TGACTTCCACAGCCAGATGGCTGTGCACAAGCTGGCCAAGAGCATCCCT
CTGCGCAGACAGGTAACAGTGTCTGCTGACTCCAGTGCATCCATGAACT
CTGGGGTTCTTCTGGTTCGGCCATCACGGCTCTCCTCCAGTGGGACTCC
CATGCTAGCAGGGGTCTCTGAGTATGAGCTTCCCGAAGACCCTCGCTGG
GAGCTGCCTCGGGACAGACTGGTCTTAGGCAAACCCCTGGGAGAGGGCT
GCTTTGGGCAGGTGGTGTGGCAGAGGCTATCGGGCTGGACAAGGACAA
ACCCAACCGTGTGACCAAAGTGGCTGTGAAGATGTTGAAGTCGGACGCA
ACAGAGAAAGACTTGTGACAGACCTGATCTCAGAAATGGAGATGATGAAGA
TGATCGGGAAGCATAAGAATATCATCAACCTGCTGGGGGCCTGCACGCA
GGATGGTCCCTTGTATGTCATCGTGGAGTATGCCTCCAAGGGCAACCTG
CGGGAGTACCTGCAGGCCCGGAGGCCCCAGGGCTGGAATACTGCTACA
ACCCAGCCACAACCCAGAGGAGCAGCTCTCCTCCAAGGACCTGGTGTG
CTGCGCCTACCAGGTGGCCCGAGGCATGGAGTATCTGGCCTCCAAGAAG
TGCATACACCGAGACCTGGCAGCCAGGAATGTCCTGGTGACAGAGGACA
ATGTGATGAAGATAGCAGACTTTGGCCTCGCACGGGACATTCACCACAT
CGACTACTATAAAAAGACAACCAACGGCCGACTGCCTGTGAAGTGGATG
GCACCCGAGGCATTATTTGACCGGATCTACACCCACCAGAGTGATGTGT
GGTCTTTCGGGGTGCTCCTGTGGGAGATCTTCACTCTGGGCGGCTCCCC
ATACCCCGGTGTGCCTGTGGAGGAACCTTTTCAAGCTGCTGAAGGAGGGT
CACCGCATGGACAAGCCCAGTAACTGCACCAACGAGCTGTACATGATGA
TGCGGGACTGCTGGCATGCAGTGCCTCACAGAGACCCACCTTCAAGCA
GCTGGTGGAAAGACCTGGACCGCATCGTGGCCTTGACCTCCAACCAGGAG
TACCTGGACCTGTCCATGCCCCTGGACCAGTACTCCCCAGCTTTCCTCG
ACACCCGGAGCTCTACGTGCTCCTCAGGGGAGGATTCCGTCTTCTCTCA
TGAGCCGCTGCCCGAGGAGCCCTGCCTGCCCGACACCCAGCCAGCTT
GCCAATGGCGGACTCAAACGCCGctgagcggccgc