

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of FEN1 [1 - 380]

<u>Enzyme description:-</u>	FEN1 [1 – 380]
<u>Clone number:-</u>	DU 40892
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	C-terminal His6
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 43, 843.69 daltons
Average Mass 43, 871.34 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.68

Purity:- >80 %

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 °C

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Clone Data Sheet

FEN1 [1 – 380]

Protein FEN1 [1 – 380]

Clone number DU 40892

Species Human

Accession number NM_004111.5

Tags C-terminal His6

Bacterially expressed protein **MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAVRQG
GDVLQNEEGETTSHLMGMFYRTIRMMENGIKPVYVFDGKPPQLKSGELA
KRSERRAEAEKQLQQAQAAGAEQEVEKFTKRLVKVTKQHNDCKHLLSL
MGIPYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGSPVLMRHLTA
SEAKKLPIQEFHLSRILQELGLNQEQFVDLCILLGSDYCESIRGIGPKR
AVDLIQKHKSIEEIVRRLDPNKYPVPENWLHKEAHQLFLEPEVLDPELV
ELKWSEPNEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLLDDF
FKVTGSLSSAKRKEPEPKGSTKKKAKTGAAGKFKRGKAAALEHHHHHHH**

Native sequence Amino acids M1 – K380 (end) of human FEN1.
Residue M1 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 386-391.

Cloning sites *Nco*1 and *Not*1 sites of pET28a

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Nucleotide
Sequence Of Insert

ATGGGAATTCAAGGCCTGGCCAAACTAATTGCTGATGTGGCCCCCAGTG
CCATCCGGGAGAATGACATCAAGAGCTACTTTGGCCGTAAGGTGGCCAT
TGATGCCTCTATGAGCATTTATCAGTTCCTGATTGCTGTTCCGCCAGGGT
GGGATGTGCTGCAGAATGAGGAGGGTGAGACCACCAGCCACCTGATGG
GCATGTTCTACCGCACCATTTCGCATGATGGAGAACGGCATCAAGCCCGT
GTATGTCTTTGATGGCAAGCCGCCACAGCTCAAGTCAGGCGAGCTGGCC
AAACGCAGTGAGCGGCGGGCTGAGGCAGAGAAGCAGCTGCAGCAGGCTC
AGGCTGCTGGGGCCGAGCAGGAGGTGGAAAAATCACTAAGCGGCTGGT
GAAGGTCACTAAGCAGCACAATGATGAGTGCAAACATCTGCTGAGCCTC
ATGGGCATCCCTTATCTTGATGCACCCAGTGAGGCAGAGGCCAGCTGTG
CTGCCCTGGTGAAGGCTGGCAAAGTCTATGCTGCGGCTACCGAGGACAT
GGACTGCCTCACCTTCGGCAGCCCTGTGCTAATGCGACACCTGACTGCC
AGTGAAGCCAAAAAGCTGCCAATCCAGGAATTCCACCTGAGCCGGATTC
TGCAGGAGCTGGGCCTGAACCAGGAACAGTTTGTGGATCTGTGCATCCT
GCTAGGCAGTGACTACTGTGAGAGTATCCGGGGTATTGGGCCCAAGCGG
GCTGTGGACCTCATCCAGAAGCACAAGAGCATCGAGGAGATCGTGCGGC
GACTTGACCCCAACAAGTACCCTGTGCCAGAAAATTGGCTCCACAAGGA
GGCTCACCAGCTCTTCTTGGAACCTGAGGTGCTGGACCCAGAGTCTGTG
GAGCTGAAGTGGAGCGAGCCAAATGAAGAAGAGCTGATCAAGTTCATGT
GTGGTGAAGAGCAGTTCTCTGAGGAGCGAATCCGCAGTGGGGTCAAGAG
GCTGAGTAAGAGCCGCCAAGGCAGCACCCAGGGCCGCCTGGATGATTTT
TTCAAGGTGACCGGCTCACTCTCTTCAGCTAAGCGCAAGGAGCCAGAAC
CCAAGGGATCCACTAAGAAGAAGGCAAAGACTGGGGCAGCAGGGAAGTT
TAAAAGGGGAAAAGCGGCCGCACTCGAGCACCACCACCACCACtga

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