

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of FEN1 R100A [1 - 380]

Enzyme description:- FEN1 R100A [1 – 380]

Clone number:- DU 40914

Source:- Recombinant

Expression system:- *E.coli*

Tag:- C-terminal His6

Purification method:- Ni²⁺-NTA agarose

Calculated molecular mass:-

Monoisotopic 43, 758.63 daltons

Average Mass 43, 786.23 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.54

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 R100A [1 – 380]

<u>Protein</u>	FEN1 R100A [1 – 380]
<u>Clone number</u>	DU 40914
<u>Species</u>	Human
<u>Accession number</u>	NM_004111.5
<u>Tags</u>	C-terminal His6
<u>Bacterially expressed protein</u>	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAVRQG GDVLQNEEGETTSHLMGMFYRTIRMMENGIKPVYVFDGKPPQLKSGELA KASERRAEAEKQLQQAQAAGAEQEVEKFTKRLVKVTKQHNDCKHLLSL MGIPYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGSPVLMRHLTA SEAKKLP IQEFHLSRILQELGLNQEQFVDLCILLGSDYCESIRGIGPKR AVDLIQKHKSIEEIVRRLDPNKYPVPENWLHKEAHQLFLEPEVLDPELV ELKWSEPNEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLLDDF FKVTGSLSSAKRKEPEPKGSTKKKAKTGAAGKFKRGKAAALEHHHHHH
<u>Native sequence</u>	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. The protein has an R100A mutation. Residue R100 is equivalent to residue A100 of the fusion protein.
<u>Cloning sites</u>	<i>Nco</i> 1 and <i>Not</i> 1 sites of pET28a

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Nucleotide
Sequence Of Insert

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

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