

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of active FES [1 – 822]

<u>Enzyme description:-</u>	FES [1 - 822]
<u>Clone number:-</u>	DU 33729
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Cobalt agarose

Calculated molecular mass:-

Monoisotopic 96, 807.62 daltons

Average Mass 96, 868.20 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.22

Purity:- 85 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 10 mM DTT, 10 mM magnesium acetate, 0.1 mM EGTA

Substrate:-

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

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Clone Data Sheet

FES [1 - 822]

Protein FES [1 – 822]

Clone number DU 33729

Species Human

Accession number P07332-1

Tags N-terminal His(6)

**Baculovirus
expressed protein**

MSYYHHHHHDYDIPTTENLYFQGAMGSMGFSSELCSPOGHGVLQOMQE
AELRLLEGMRKWMAQRVKS DREYAGLLHHMSLQDSGGQSR AISPDSPIS
QSWAEITSQTEGLSRLLRQHAEDLNSGPLSKLSLLIRERQQLRKTYSEQ
WQQLQQELTKTHSQDIEKLSQYRALARDSAQAKRKYQEASKDKDRDKA
KDKYVRSLWKLFAHNNRYVLGVRAAQLHHQHQQHLLPGLLRSLQDLHE
EMACILKEILQEYLEISSLVQDEVVAIHREMAAAAARIQPEAEYQGFLR
QYGSAPDVPPCVTFDESLL EEGEPLEPGELQLNELTVESVQHTLTSVTD
ELAVATEMVFRQEMVTQLQQELRNEEENTHPRERVQLLGKRQVLQEAL
QGLQVALCSQAKLQAQQELLQTKLEHLGPGEPPPVL LLQDDRHSSTSSSE
QEREGGRTP TLEILKSHISGIFRPKFS LPPPLQLIPEVQKPLHEQLWYH
GAIPRAEVAELLVHSGDFLVRESQ GKQYVLSVLWDGLPRHFIIQSLDN
LYRLEGEGFPSIPLLIDHLLSTQQPLTKKSGVV LHRAVPKDKWVLN HED
LVLGEQIGRGNFGEVFSGRRLRADNTLVAVKSCRETLP PDLKAKFLQEAR
ILKQYSHPNIVRLIGVCTQKQPIYIVMELVQGGDFLTFLRTEGARLRVK
TLLQMVGDAAAGMEYLESKCCIHRDLAARNCLVTEKNVLKISDFGMSRE
EADGVYAASGGLRQVPVKWTAPEALNYGRYSSES DVWSFGILLWETFSL
GASPYPNLSNQOTREFVEKGGRLPCPELCPDAVFRLMEQCWAYEPGQRP
SFSTIYQELQSIRKRRH

Native sequence Amino acids M1 – R822 (end) of human FES.
Residue M29 of the fusion protein is equivalent to M1 of the native
enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQ) residues 18 - 23

Cloning sites *Bgl*II and *Not*I sites of pFastBac.Htb

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Nucleotide Sequence Of Insert

agatctATGGGCTTCTCTTCCGAGCTGTGCAGCCCCCAGGGCCACGGGGTCCTGCAGCAAATGCAG
GAGGCCGAGCTTCGTCTACTGGAGGGCATGAGAAAGTGGATGGCCAGCGGGTCAAGAGTGACAGG
GAGTATGCAGGACTGCTTCACCACATGTCCCTGCAGGACAGTGGGGGCCAGAGCCGGGCCATCAGC
CCTGACAGCCCCATCAGTCAGTCCTGGGCTGAGATCACCAGCCAAACTGAGGGCCTGAGCCGCTTG
CTGCGGCAGCACGCAGAGGATCTGAACTCAGGGCCCCCTGAGCAAGCTGAGCCTGCTCATCCGGGAA
CGGCAGCAGCTTCGCAAGACCTACAGCGAGCAGTGGCAGCAGCTGCAGCAGGAGCTCACCAAGACC
CACAGCCAGGACATTGAGAAGCTGAAGAGCCAGTACCGAGCTCTGGCACGGGACAGTGCCCAAGCC
AAGCGCAAGTACCAGGAGGGCCAGCAAAGACAAGGACCGTGACAAGGCCAAGGACAAGTATGTGCGC
AGCCTGTGGAAGCTCTTTGCTCACCACAACCGCTATGTGCTGGGCGTGCGGGCTGCGCAGCTACAC
CACCAGCACCACCAGCTCCTGCTGCCCCGGCCTGCTGCGGTCACTGCAGGACCTGCACGAGGAG
ATGGCTTGCATCCTGAAGGAGATCCTGCAGGAATACCTGGAGATTAGCAGCCTGGTGCAGGATGAG
GTGGTGGCCATTACCGGGAGATGGCTGCAGCTGCTGCCCGCATCCAGCCTGAGGCTGAGTACCAA
GGCTTCTGCGACAGTATGGGTCCGCACCTGACGTCCACCCCTGTGTACGTTCGATGAGTCACTG
CTTGAGGAGGGTGAACCGCTGGAGCCTGGGGAGCTCCAGCTGAACGAGCTGACTGTGGAGAGCGTG
CAGCACACGCTGACCTCAGTGACAGATGAGCTGGCTGTGGCCACCGAGATGGTGTTTCAGGCGGCAG
GAGATGGTTACGCAGCTGCAACAGGAGCTCCGGAATGAAGAGGAGAACACCCACCCCCGGGAGCGG
GTGCAGCTGCTGGGCAAGAGGCAAGTGCTGCAAGAAGCACTGCAGGGGCTGCAGGTAGCGCTGTGC
AGCCAGGCCAAGCTGCAGGCCCAGCAGGAGTTGCTGCAGACCAAGCTGGAGCACCTGGGCCCCGGC
GAGCCCCCGCCTGTGCTGCTCCTGCAGGATGACCGCCACTCCACGTCGTCTCTCGGAGCAGGAGCGA
GAGGGGGGAAGGACACCCACGCTGGAGATCCTTAAGAGCCACATCTCAGGAATCTTCCGCCCCAAG
TTCTCGCTCCCTCCACCGCTGCAGCTCATTCGGAGGTGCAGAAGCCCCCTGCATGAGCAGCTGTGG
TACCACGGGGCCATCCCGAGGGCAGAGGTGGCTGAGCTGCTGGTGCCTCTGGGGACTTCCTGGTG
CGGGAGAGCCAGGGCAAGCAGGAGTACGTGCTGTGCGTGCTGTGGGATGGTCTGCCCCGGCACTTC
ATCATCCAGTCTTTGGATAACCTGTACCGACTGGAAGGGGAAGGCTTTCCTAGCATTCCTTTGCTC
ATCGACCACCTACTGAGCACCCAGCAGCCCCCTACCAAGAAGAGTGGTGTTGTCCTGCACAGGGCT
GTGCCCAAGGACAAGTGGGTGCTGAACCATGAGGACCTGGTGTTGGGTGAGCAGATTGGACGGGGG
AACTTTGGCGAAGTGTTACGCGGACGCCTGCGAGCCGACAACACCCTGGTGGCGGTGAAGTCTTGT
CGAGAGACGCTCCACCTGACCTCAAGGCCAAGTTTCTACAGGAAGCGAGGATCCTGAAGCAGTAC
AGCCACCCCAACATCGTGCGTCTCATTGGTGTCTGCACCCAGAAGCAGCCCATCTACATCGTCATG
GAGCTTGTGCAGGGGGGCGACTTCCTGACCTTCCTCCGCACGGAGGGGGCCCGCCTGCGGGTGAAG
ACTCTGCTGCAGATGGTGGGGGATGCAGCTGCTGGCATGGAGTACCTGGAGAGCAAGTGTGCATC
CACCGGGACCTGGCTGCTCGGAAGTGCCTGGTGACAGAGAAGAATGTCCTGAAGATCAGTGACTTT
GGGATGTCCCGAGAGGAAGCCGATGGGGTCTATGCAGCCTCAGGGGGCCTCAGACAAGTCCCCGTG
AAGTGGACCGCACCTGAGGCCCTTAACACGGCCGCTACTCCTCCGAAAGCGACGTGTGGAGCTTT
GGCATCTTGCTCTGGGAGACCTTCAGCCTGGGGGCCTCCCCCTATCCCAACCTCAGCAATCAGCAG
ACACGGGAGTTTGTGGAGAAGGGGGGCGTCTGCCCTGCCAGAGCTGTGTCCTGATGCCGTGTTT
AGGCTCATGGAGCAGTGCTGGGCCTATGAGCCTGGGCAGCGGCCAGCTTCAGCACCATCTACCAG
GAGCTGCAGAGCATCCGAAAGCGGCATCGGTgagcgccgc