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Standard Operating Procedure

Preparation of active ERK8 [2 - 544]

<u>Enzyme description:-</u>	ERK8 [2 - 544]
<u>Clone number:-</u>	DU 662
<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>	3 - 4 mg/L
<u>Calculated molecular mass:-</u>	62, 620 daltons
<u>Purity:-</u>	75 %
<u>Activation protocol:-</u>	Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

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:-

Myelin basic protein Final concentration: 0.3 mg/ml

Specific activity range:- 60 – 120 U/mg

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Clone Data Sheet - ERK8 [2 - 544]

Protein ERK8 [2 - 544]

Clone number DU 662

Species Human

Accession number AY065978

Tags N-terminal His(6)

Baculovirus-expressed protein MHHHHHDYDIPTTENLYFOGAMGSC**TVVDPRI**VRRYLLRRQLGQGAYG
IVWKA**VDRRTGEVVAIKKIFDAFRDKTDAQRTFREITLLQEF**GDHPNI I
SLLDVIRAENDRDIYLVFEFMDTDLNAVIRK**GGLLQDVH**VRSIFYQLLR
AT**FLHSGHV**VHRDQKPSNVLLDANCTVKLCDFGLARS**LGDLPEG**PEDQ
AVTEYVATRWYRAPEVLLSSHRYTLGVDMW**SLGCILGEM**LRGRPLFPGT
STLH**QLELILE**TIPPPSEEDLLALGSGCRASVLH**QLGSR**PRQTLDALLP
PDT**SPEALDLLRRL**LVFAPDKRLSATQALQHPYV**QRFHC**PSDEWAREAD
VR**PRAHEGV**QLSVPEYRSRVYQ**MILECGSSG**TSREK**GPEGV**SPSQ**AHL**
HKPRAD**PQLPSRTPVQ**GPRPRPQSSPGHDP**AEHES**PRAAKNVPRQNSAP
LLQ**TALLNGER**PPGAKEAPPLTSLVKPSGRGAAPSL**TSQAAAQ**VANQ
ALIRGDWNRGGGVRVASVQ**QVPPRLP**PEARPGRRMF**STSALQGA**QGGAR
ALLGGYSQAYGTVCHSALGHLPLLEG**HV**

Native sequence Amino acids C2 – V544 (end) of human ERK8.
Residue C26 of the fusion protein is equivalent to C2 of the native enzyme. The His(6) tag is located at residues 2 – 7 of the fusion protein.

Protease cleavage rTEV (**ENLYFOG**) residues 15 - 21

Cloning sites *Bam*H1 and *Eco*R1 site of pFastBAC HTb

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**Nucleotide
sequence of insert**

GGATCCTGCACCGTAGTGGACCCTCGCATTGTCCGGAGATACCTACTCA
GGCGGCAGCTCGGGCAGGGGGCCTATGGCATTGTGTGGAAGGCAGTGGA
CCGGAGGACTGGTGAGGTCGTGGCCATCAAGAAAATCTTTGATGCTTTT
AGGGATAAGACAGATGCCAGAGAACATTCGGGAAATCACGCTCCTCC
AGGAGTTTGGGGACCATCCCAACATCATCAGCCTCCTTGACGTGATCCG
GGCAGAGAACGACAGGGACATTTACCTGGTGTGTTGAGTTTATGGACACT
GACCTGAACGCAGTCATCCGGAAGGGCGGCCTGCTGCAGGACGTCCACG
TGCCTCCATCTTCTACCAGCTCCTGCGGGCCACCCGGTTCCTCCACTC
GGGGCACGTTGTGCACCGGGACCAGAAGCCGTCCAATGTGCTCCTGGAT
GCCAACTGCACAGTGAAGCTGTGTGACTTTGGCCTGGCCCCGCTCCCTGG
GCGACCTCCCTGAGGGGCCTGAGGACCAGGCCGTGACAGAGTACGTGGC
CACACGCTGGTACCGAGCACCGGAGGTGCTGCTCTCTTCGCACCGATAC
ACCCTTGGGGTGGACATGTGGAGTCTGGGCTGTATCCTGGGGGAGATGC
TGCGGGGGAGACCCCTGTTCCCCGGCACGTCCACCCTCCACCAGCTGGA
GCTGATCCTGGAGACCATCCACCGCCATCTGAGGAGGACCTCCTGGCT
CTCGGCTCAGGCTGCCGTGCCTCTGTGCTGCACCAGCTGGGGTCCCGGC
CACGACAGACGCTGGATGCCCTCCTACCGCCAGACACCTCCCCAGAGGC
CTTGACCTCCTTAGGCGACTCCTGGTGTTCGCCCCGGACAAGCGGTTA
AGCGCGACCCAGGCACTGCAGCACCCCTACGTGCAGAGGTTCCACTGCC
CCAGCGACGAGTGGGCACGAGAGGCAGATGTGCGGGCCCCGGGCACACGA
AGGGGTCCAGCTCTCTGTGCCTGAGTACCGCAGCCGCGTCTATCAGATG
ATCCTGGAGTGTGGAGGCAGCAGCGGCACCTCGAGAGAGAAGGGCCCGG
AGGGTGTCTCCCCAAGCCAGGCACACCTGCACAAACCCAGAGCCGACCC
TCAGCTGCC'TTCTAGGACACCTGTGCAGGGTCCCAGACCCAGGCCCCAG
AGCAGCCCAGGCCATGACCCTGCCGAGCACGAGTCCCCCGTGCAGCCA
AGAACGTTCCAGGCAGAACTCCGCTCCCCTGCTCCAAACTGCTCTCCT
AGGGAATGGGGAAAGGCCCCCTGGGGCGAAGGAAGCGCCCCCTTGACA
CTCTCGCTGGTGAAGCCAAGCGGGAGGGGAGCTGCGCCCTCCCTGACCT
CCCAGGCTGCGGCTCAGGTGGCCAACCAGGCCCTGATCCGGGGTGACTG
GAACCGGGGCGGTGGGGTGAAGGTGGCCAGCGTACAACAGGTCCCTCCC
CGGCTTCTCCGGAGGCCCGGCCCGGCGGAGGATGTTTACGACCTCTG
CCTTGCAGGGTGCCAGGGGGGTGCCAGGGCTTTGCTTGAGGCTACTC
CCAAGCCTACGGGACTGTCTGCCACTCGGCACTGGGCCACCTGCCCTG
CTGGAGGGGCACCATGTGtgagaattc