Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active ERK8 [2 - 544]

Enzyme description:- ERK8 [2 - 544]

Clone number:- DU 662

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 3 - 4 mg/L

Calculated molecular mass:- 62, 620 daltons

Purity:- 75 %

Activation protocol:- 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	MHHHHHHDYDIPTTENLYFQGAMGS**CTVVDPRIVRRYLLRRQLGQGAYG IVWKAVDRRTGEVVAIKKIFDAFRDKTDAQRTFREITLLQEFGDHPNII SLLDVIRAENDRDIYLVFEFMDTDLNAVIRKGGLLQDVHVRSIFYQLLR ATRFLHSGHVVHRDQKPSNVLLDANCTVKLCDFGLARSLGDLPEGPEDQ AVTEYVATRWYRAPEVLLSSHRYTLGVDMWSLGCILGEMLRGRPLFPGT STLHQLELILETIPPPSEEDLLALGSGCRASVLHQLGSRPRQTLDALLP PDTSPEALDLLRRLLVFAPDKRLSATQALQHPYVQRFHCPSDEWAREAD VRPRAHEGVQLSVPEYRSRVYQMILECGGSSGTSREKGPEGVSPSQAHL HKPRADPQLPSRTPVQGPRPRPQSSPGHDPAEHESPRAAKNVPRQNSAP LLQTALLGNGERPPGAKEAPPLTLSLVKPSGRGAAPSLTSQAAAQVANQ ALIRGDWNRGGGVRVASVQQVPPRLPPEARPGRRMFSTSALQGAQGGAR ALLGGYSQAYGTVCHSALGHLPLLEGHHV**
Native sequence	Amino acids C2 – V544 (end) of human ERK8. Residue C26 of the fusion protein is equilivalent to C2 of the native enzyme. The His(6) tag is located at residues 2 – 7 of the fusion protein.
Protease cleavage	rTEV (ENLYFQG) residues 15 - 21
Cloning sites	*Bam*H1 and *Eco*R1 site of pFastBAC HTb

Nucleotide sequence of insert

GGATCCTGCACCGTAGTGGACCCTCGCATTGTCCGGAGATACCTACTCA
GGCGGCAGCTCGGGCAGGGGGCCTATGGCATTGTGTGGAAGGCAGTGGA
CCGGAGGACTGGTGAGGTCGTGGCCATCAAGAAAATCTTTGATGCTTTT
AGGGATAAGACAGATGCCCAGAGAACATTCCGGGAAATCACGCTCCTCC
AGGAGTTTGGGGACCATCCCAACATCATCAGCCTCCTTGACGTGATCCG
GGCAGAGAACGACAGGGACATTTACCTGGTGTTTGAGTTTATGGACACT
GACCTGAACGCAGTCATCCGGAAGGGCGGCCTGCTGCAGGACGTCCACG
TGCGCTCCATCTTCTACCAGCTCCTGCGGGCCACCCGGTTCCTCCACTC
GGGGCACGTTGTGCACCGGGACCAGAAGCCGTCCAATGTGCTCCTGGAT
GCCAACTGCACAGTGAAGCTGTGTGACTTTGGCCTGGCCCGCTCCCTGG
GCGACCTCCCTGAGGGGCCTGAGGACCAGGCCGTGACAGAGTACGTGGC
CACACGCTGGTACCGAGCACCGGAGGTGCTGCTCTCTTCGCACCGATAC
ACCCTTGGGGTGGACATGTGGAGTCTGGGCTGTATCCTGGGGGAGATGC
TGCGGGGGAGACCCCTGTTCCCCGGCACGTCCACCCTCCACCAGCTGGA
GCTGATCCTGGAGACCATCCCACCGCCATCTGAGGAGGACCTCCTGGCT
CTCGGCTCAGGCTGCCGTGCCTCTGTGCTGCACCAGCTGGGGTCCCGGC
CACGACAGACGCTGGATGCCCTCCTACCGCCAGACACCTCCCCAGAGGC
CTTGGACCTCCTTAGGCGACTCCTGGTGTTCGCCCCGGACAAGCGGTTA
AGCGCGACCCAGGCACTGCAGCACCCCTACGTGCAGAGGTTCCACTGCC
CCAGCGACGAGTGGGCACGAGAGGCAGATGTGCGGCCCCGGGCACACGA
AGGGGTCCAGCTCTCTGTGCCTGAGTACCGCAGCCGCGTCTATCAGATG
ATCCTGGAGTGTGGAGGCAGCAGCGGCACCTCGAGAGAGAAGGGCCCGG
AGGGTGTCTCCCCAAGCCAGGCACACCTGCACAAACCCAGAGCCGACCC
TCAGCTGCCTTCTAGGACACCTGTGCAGGGTCCCAGACCCAGGCCCCAG
AGCAGCCCAGGCCATGACCCTGCCGAGCACGAGTCCCCCCGTGCAGCCA
AGAACGTTCCCAGGCAGAACTCCGCTCCCCTGCTCCAAACTGCTCTCCT
AGGGAATGGGGAAAGGCCCCCTGGGGCGAAGGAAGCGCCCCCCTTGACA
CTCTCGCTGGTGAAGCCAAGCGGGAGGGGAGCTGCGCCCTCCCTGACCT
CCCAGGCTGCGGCTCAGGTGGCCAACCAGGCCCTGATCCGGGGTGACTG
GAACCGGGGCGGTGGGGTGAGGGTGGCCAGCGTACAACAGGTCCCTCCC
CGGCTTCCTCCGGAGGCCCGGCCCGGCCGGAGGATGTTCAGCACCTCTG
CCTTGCAGGGTGCCCAGGGGGGTGCCAGGGCTTTGCTTGGAGGCTACTC
CCAAGCCTACGGGACTGTCTGCCACTCGGCACTGGGCCACCTGCCCCTG
CTGGAGGGGCACCATGTGtgagaattc