

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ERK5 [1 – 816]

<u>Enzyme description:-</u>	ERK5 [1 – 816]
<u>Clone number:-</u>	DU 842
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	89, 284.48 daltons
Average Mass	89, 340.51 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.81
<u>Purity:-</u>	80 %
<u>Activation protocol:-</u>	Co-expression with GST-MKK5 S311D T315D
<u>Enzyme storage buffer:-</u>	
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	
<u>Storage temperature:-</u>	-70 °C
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
Myelin Basic Protein (MBP)	Final concentration: 1 mg/ml

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Clone Data Sheet

ERK5 [1 – 816]

Protein ERK5 [1 - 816]

Clone number DU 842

Species Human

Accession number NM_002749

Tags N-terminal His(6)

**Baculovirus
expressed protein**

MHHHHHHMAEPLKEEDGEDGSAEPPGPVKAEPHAHTAASVAAKNLA
LLKARSFDVTFDVGDEYEI IETIGNGAYGVVSSARRRLTGQOVAI
KKIPNAFDVVTNAKRTLRELKILKHFKHDNIIA IKDILRPTVPYG
EFKSVYVVLDMESDLHQI IHSSQPLTLEHVRYFLYQLLRGLKYM
HSAQVIHRDLKPSNLLVNENCELKIGDFGMARGLCTSPAHEQYFM
TEYVATRWRAPELMLSLHEYTQ AIDLWSVGCIFGEMLARRQLFP
GKNYVHQQLIMMVLGTPSPAVIQAVGAERVRAIYQSLPPROPVP
WETVYPGADRQALSLLGRMLRFEPSARI SAAAALRHPFLAKYHDP
DDEPDCAPPFDFAFDREALTRERIKEAIVAEIEDFHARREGIRQQ
IRFQPSLQPVASEPGCPDVEPSPWAPSGDCAMESPPPAPPPCPG
PAPDTIDLTLQPPPPVSEPPPKDGAISDNTKAALKAALLKSLR
SRLRDGPSAPLEAPEPRKPVTAQERQREEREKRRRRQERAKEREK
RRQERERKERGAGASGGPSTDPLAGLVLSDNDRSLLERWTRMARP
AAPALTSVPAPAPAPTPTPTPVQPTSPPPGPVAQPTGPQPOSAGS
TSGPVPQPACPPPGPAPHPTGPPGPIPVPPAPPQIATSTSLAAQS
LVPPPGLPGSSTPGVLPYFPPGLPPPDAGGAPQSSMSESPDVNLV
TQQLSKSQVEDPLPPVFSGTPKGSAGYGVGFDLEEFNLQSFDMG
VADGPQDQADSASLSASLLADWLEGHGMNPADIESLQREIQMDS
PMLLADLPDLQDP

Native sequence Amino acids M1 – P816 (end) of human ERK5.
Residue M8 of the fusion protein is equivalent to M1 of the native
enzyme. The His6 tag is located at residues 2 - 7.

Protease cleavage None

Cloning sites *Bam*H1 and *Sal*I sites of pFastBac1

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**Nucleotide
sequence of insert**

ggatccccATGCACCATCACCATCACCATATGGCCGAGCCTCTGA
AGGAGGAAGACGGCGAGGACGGCTCTGCGGAGCCCCCGGGCCCG
TGAAGGCCGAACCCGCCACACCGCTGCCTCTGTAGCGGCCAAGA
ACCTGGCCCTGCTTAAAGCCCGCTCCTTCGATGTGACCTTTGACG
TGGGCGACGAGTACGAGATCATCGAGACCATAGGCAACGGGGCCT
ATGGAGTGGTGTCTCCGCCCCGCCGCCCTCACCGGCCAGCAGG
TGGCCATCAAGAAGATCCCTAATGCTTTCGATGTGGTGACCAATG
CCAAGCGGACCCTCAGGGAGCTGAAGATCCTCAAGCACTTTAAAC
ACGACAACATCATCGCCATCAAGGACATCCTGAGGCCACCGTGC
CCTATGGCGAATTCAAATCTGTCTACGTGGTCCCTGGACCTGATGG
AAAGCGACCTGCACCAGATCATCCACTCCTCACAGCCCCTCACAC
TGGAACACGTGCGTACTTCCTGTACCAACTGCTGCGGGGCTGA
AGTACATGCACTCGGCTCAGGTCATCCACCGTGACCTGAAGCCCT
CCAACCTATTGGTGAATGAGAAGTGTGAGCTCAAGATTGGTGACT
TTGGTATGGCTCGTGGCCTGTGCACCTCGCCCGCTGAACATCAGT
ACTTCATGACTGAGTATGTGGCCACGCGTGGTACCGTGCGCCCG
AGCTCATGCTCTCTTTGCATGAGTATACACAGGCTATTGACCTCT
GGTCTGTGGGCTGCATCTTTGGTGAGATGCTGGCCCCGGCGCCAGC
TCTTCCCAGGCAAAAATACTATGTACACCAGCTACAGCTCATCATGA
TGGTGCTGGGTACCCCATCACCAGCCGTGATTCAGGCTGTGGGGG
CTGAGAGGGTGCGGGCCTATATCCAGAGCTTGCCACCACGCCAGC
CTGTGCCCTGGGAGACAGTGTACCCAGGTGCCGACCGCCAGGCC
TATCACTGCTGGGTGCGATGCTGCGTTTTGAGCCAGCGCTCGCA
TCTCAGCAGCTGCTGCCCTTCGCCACCCTTTTCTGGCCAAGTACC
ATGATCCTGATGATGAGCCTGACTGTGCCCCGCCCTTTGACTTTG
CCTTTGACCGCGAAGCCCTCACTCGGGAGCGCATTAAGGAGGCCA
TTGTGGCTGAAATTGAGGACTTCCATGCAAGGCGTGAGGGCATCC
GCCAACAGATCCGCTTCCAGCCTTCTCTACAGCCTGTGGCTAGTG
AGCCTGGCTGTCCAGATGTTGAAATGCCAGTCCCTGGGCTCCCA
GTGGGGACTGTGCCATGGAGTCTCCACCACCAGCCCCGCCACCAT
GCCCCGGCCCTGCACCTGACACCATTGATCTGACCCTGCAGCCAC
CTCCACCAGTCAGTGAGCCTGCCCCACCAAGAAAGATGGTGCCA
TCTCAGACAATACTAAGGCTGCCCTTAAAGCTGCCCTGCTCAAGT
CTTTGAGGAGCCGGCTCAGAGATGGCCCCAGCGCACCCCTGGAGG
CTCCTGAGCCTCGGAAGCCGGTGACAGCCAGGAGCGCCAGCGGG
AGCGGGAGGAGAAGCGGCGGAGGCGGCAAGAACGAGCCAAGGAGC
GGGAGAAACGGCGGCAGGAGCGGGAGCGAAAGGAACGGGGGGCTG
GGCCTCTGGGGGCCCTCCACTGACCCCTTTGGCTGGACTAGTGC
TCAGTGACAATGACAGAAGCCTGTTGGAACGCTGGACTCGAATGG
CCCGGCCCGCAGCCCCAGCCCTCACCTCTGTGCCGGCCCCCTGCC
CAGCGCCAACGCCAACCCCAACCCAGTCCAACCTACCAGTCCTC
CTCCTGGCCCTGTAGCCAGCCCACTGGCCCCGCAACCACAATCTG

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CGGGCTCTACCTCTGGCCCTGTACCCCAGCCTGCCTGCCACCCC
CTGGCCCTGCACCCCACCCACTGGCCCTCCTGGGCCATCCCTG
TCCCCGCGCCACCCAGATTGCCACCTCCACCAGCCTCCTGGCTG
CCCAGTCACTTGTGCCACCCCTGGGCTGCCTGGCTCCAGCACCC
CAGGAGTTTTGCCTTACTTCCCACCTGGCCTGCCGCCCCAGACG
CCGGGGGAGCCCCTCAGTCTTCCATGTCAGAGTCACCTGATGTCA
ACCTTGTGACCCAGCAGCTATCTAAGTCACAGGTGGAGGACCCCC
TGCCCCCTGTGTTCTCAGGCACACCAAAGGGCAGTGGGGCTGGCT
ACGGTGTGGCTTTGACCTGGAGGAATTCCTAAACCAGTCTTTCG
ACATGGGCGTGGCTGATGGGCCACAGGATGGCCAGGCAGATTCAG
CCTCTCTCTCAGCCTCCCTGCTTGCTGACTGGCTCGAAGGCCATG
GCATGAACCCTGCCGATATTGAGTCCCTGCAGCGTGAGATCCAGA
TGGACTCCCCAATGCTGCTGGCTGACCTGCCTGACCTCCAGGACC
CCtgagtcgac