*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of active ERK5 [1 – 816]

**Enzyme description:-** ERK5 [1 – 816]

**Clone number:-** DU 842

**Source:-** Recombinant

**Expression system:-** Baculovirus expression vector system

**Tag:-** N-terminal His(6) tag

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

**Calculated molecular mass:-**
Monoisotopic 89, 284.48 daltons
Average Mass 89, 340.51 daltons
[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 5.81

**Purity:-** 80 %

**Activation protocol:-** Co-expression with GST-MKK5 S311D T315D

**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

**Assay buffer:-**
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

**Substrate:-**
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** | MHHHHHH**MAEPLKEEDGEDGSAEPPGPVKAEPAHTAASVAAKNLA LLKARSFDVTFDVGDEYEIIETIGNGAYGVVSSARRRLTGQQVAI KKIPNAFDVVTNAKRTLRELKILKHFKHDNIIAIKDILRPTVPYG EFKSVYVVLDLMESDLHQIIHSSQPLTLEHVRYFLYQLLRGLKYM HSAQVIHRDLKPSNLLVNENCELKIGDFGMARGLCTSPAEHQYFM TEYVATRWYRAPELMLSLHEYTQAIDLWSVGCIFGEMLARRQLFP GKNYVHQLQLIMMVLGTPSPAVIQAVGAERVRAYIQSLPPRQPVP WETVYPGADRQALSLLGRMLRFEPSARISAAAALRHPFLAKYHDP DDEPDCAPPFDFAFDREALTRERIKEAIVAEIEDFHARREGIRQQ IRFQPSLQPVASEPGCPDVEMPSPWAPSGDCAMESPPPAPPPCPG PAPDTIDLTLQPPPPVSEPAPPKKDGAISDNTKAALKAALLKSLR SRLRDGPSAPLEAPEPRKPVTAQERQREREEKRRRRQERAKEREK RRQERERKERGAGASGGPSTDPLAGLVLSDNDRSLLERWTRMARP AAPALTSVPAPAPAPTPTPTPVQPTSPPPGPVAQPTGPQPQSAGS TSGPVPQPACPPPGPAPHPTGPPGPIPVPAPPQIATSTSLLAAQS LVPPPGLPGSSTPGVLPYFPPGLPPPDAGGAPQSSMSESPDVNLV TQQLSKSQVEDPLPPVFSGTPKGSGAGYGVGFDLEEFLNQSFDMG VADGPQDGQADSASLSASLLADWLEGHGMNPADIESLQREIQMDS PMLLADLPDLQDP** |
| **Native sequence** | Amino acids M1 – P816 (end) of human ERK5.<br>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*1 sites of pFastBac1 |

**Nucleotide sequence of insert**

ggatccccATGCACCATCACCATCACCATATGGCCGAGCCTCTGA
AGGAGGAAGACGGCGAGGACGGCTCTGCGGAGCCCCCCGGGCCCG
TGAAGGCCGAACCCGCCCACACCGCTGCCTCTGTAGCGGCCAAGA
ACCTGGCCCTGCTTAAAGCCCGCTCCTTCGATGTGACCTTTGACG
TGGGCGACGAGTACGAGATCATCGAGACCATAGGCAACGGGGCCT
ATGGAGTGGTGTCCTCCGCCCGCCGCCGCCTCACCGGCCAGCAGG
TGGCCATCAAGAAGATCCCTAATGCTTTCGATGTGGTGACCAATG
CCAAGCGGACCCTCAGGGAGCTGAAGATCCTCAAGCACTTTAAAC
ACGACAACATCATCGCCATCAAGGACATCCTGAGGCCCACCGTGC
CCTATGGCGAATTCAAATCTGTCTACGTGGTCCTGGACCTGATGG
AAAGCGACCTGCACCAGATCATCCACTCCTCACAGCCCCTCACAC
TGGAACACGTGCGCTACTTCCTGTACCAACTGCTGCGGGGCCTGA
AGTACATGCACTCGGCTCAGGTCATCCACCGTGACCTGAAGCCCT
CCAACCTATTGGTGAATGAGAACTGTGAGCTCAAGATTGGTGACT
TTGGTATGGCTCGTGGCCTGTGCACCTCGCCCGCTGAACATCAGT
ACTTCATGACTGAGTATGTGGCCACGCGCTGGTACCGTGCGCCCG
AGCTCATGCTCTCTTTGCATGAGTATACACAGGCTATTGACCTCT
GGTCTGTGGGCTGCATCTTTGGTGAGATGCTGGCCCGGCGCCAGC
TCTTCCCAGGCAAAAACTATGTACACCAGCTACAGCTCATCATGA
TGGTGCTGGGTACCCCATCACCAGCCGTGATTCAGGCTGTGGGGG
CTGAGAGGGTGCGGGCCTATATCCAGAGCTTGCCACCACGCCAGC
CTGTGCCCTGGGAGACAGTGTACCCAGGTGCCGACCGCCAGGCCC
TATCACTGCTGGGTCGCATGCTGCGTTTTGAGCCCAGCGCTCGCA
TCTCAGCAGCTGCTGCCCTTCGCCACCCTTTCCTGGCCAAGTACC
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CCTTTGACCGCGAAGCCCTCACTCGGGAGCGCATTAAGGAGGCCA
TTGTGGCTGAAATTGAGGACTTCCATGCAAGGCGTGAGGGCATCC
GCCAACAGATCCGCTTCCAGCCTTCTCTACAGCCTGTGGCTAGTG
AGCCTGGCTGTCCAGATGTTGAAATGCCCAGTCCCTGGGCTCCCA
GTGGGGACTGTGCCATGGAGTCTCCACCACCAGCCCCGCCACCAT
GCCCCGGCCCTGCACCTGACACCATTGATCTGACCCTGCAGCCAC
CTCCACCAGTCAGTGAGCCTGCCCCACCAAAGAAAGATGGTGCCA
TCTCAGACAATACTAAGGCTGCCCTTAAAGCTGCCCTGCTCAAGT
CTTTGAGGAGCCGGCTCAGAGATGGCCCCAGCGCACCCCTGGAGG
CTCCTGAGCCTCGGAAGCCGGTGACAGCCCAGGAGCGCCAGCGGG
AGCGGGAGGAGAAGCGGCGGAGGCGGCAAGAACGAGCCAAGGAGC
GGGAGAAACGGCGGCAGGAGCGGGAGCGAAAGGAACGGGGGGCTG
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TCAGTGACAATGACAGAAGCCTGTTGGAACGCTGGACTCGAATGG
CCCGGCCCGCAGCCCCAGCCCTCACCTCTGTGCCGGCCCCTGCCC
CAGCGCCAACGCCAACCCCAACCCCAGTCCAACCTACCAGTCCTC
CTCCTGGCCCTGTAGCCCAGCCCACTGGCCCGCAACCACAATCTG

CGGGCTCTACCTCTGGCCCTGTACCCCAGCCTGCCTGCCCACCCC
CTGGCCCTGCACCCCACCCCACTGGCCCTCCTGGGCCCATCCCTG
TCCCCGCGCCACCCCAGATTGCCACCTCCACCAGCCTCCTGGCTG
CCCAGTCACTTGTGCCACCCCCTGGGCTGCCTGGCTCCAGCACCC
CAGGAGTTTTGCCTTACTTCCCACCTGGCCTGCCGCCCCCAGACG
CCGGGGGAGCCCCTCAGTCTTCCATGTCAGAGTCACCTGATGTCA
ACCTTGTGACCCAGCAGCTATCTAAGTCACAGGTGGAGGACCCCC
TGCCCCCTGTGTTCTCAGGCACACCAAAGGGCAGTGGGGCTGGCT
ACGGTGTTGGCTTTGACCTGGAGGAATTCTTAAACCAGTCTTTCG
ACATGGGCGTGGCTGATGGGCCACAGGATGGCCAGGCAGATTCAG
CCTCTCTCTCAGCCTCCCTGCTTGCTGACTGGCTCGAAGGCCATG
GCATGAACCCTGCCGATATTGAGTCCCTGCAGCGTGAGATCCAGA
TGGACTCCCCAATGCTGCTGGCTGACCTGCCTGACCTCCAGGACC
CCtgagtcgac