

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active BRSK 1 [2 - 778]

<u>Enzyme description:-</u>	BRSK1 [2 - 778]
<u>Clone number:-</u>	DU 1270
<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>	2 mg/L

Calculated molecular mass:-

Monoisotopic 88, 272.15 daltons
Average Mass 88, 327.19 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.24

Purity:- >80 %

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, 0.2 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:-

CHKtide [KKKVSRSGLYRSPSPENLNRPR] Final concentration: 300 μ M

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Clone Data Sheet

BRSK1 [2 - 778]

Protein BRSK1 [2 - 778]

Clone number DU 1270

Species Human

Accession number NM_032430

Tags N-terminal His(6)

Baculovirus expressed protein MSY~~Y~~HHHHHHHDYDIPTTENLYFQ~~G~~AMGSSSSGAKEGGGGSPAYHLPHPH
HPPQHAQYVGPYRLEKTLGKGQTGLVKLGVHCITGQKVAIKIVNREKLS
ESVLMKVEREIAILKLI~~E~~HPHVLKLHDVYENKKYLVLVLEHVS~~G~~GELFD
YLVKKGRLTPKEARKFFRQIVSALDFCHSYSICH~~R~~DLKPENLLLDEKNN
IRIADFGMASLQVGDSLLETSCGSPHYACPEVIKGEKYDGRRADMWSCG
VILFALLV~~G~~ALPFDDNLRQLLEKVKRGV~~F~~HMPHFIPDCQSLLRGMIE
VEPEKRLSLEQIQKHPWYLGGKHEPDP~~C~~LEPAPGRRVAMRSLPSNGELD
PDVLES~~M~~ASLGC~~F~~RD~~R~~ERLHREL~~R~~SEEENQEKMIY~~L~~LLDRKERYPSCE
DQDLPPRNDVDP~~P~~PKRV~~D~~SPMLSRHGKRRPERKSMEVLSITDAGGGGSP
VPTRRALEMAQHSQRSRSVSGASTGLSSSPLSSPRSPVFSFSPEPGAGD
EARGGGSP~~T~~SKTQTLPSR~~G~~PRGGGAGEQPPPPSARSTPLPGPPGSPRSS
GGT~~P~~LHSP~~L~~H~~T~~PRASPTGT~~P~~GTTPPPSPGGGVGGA~~A~~WRSRLNSIRNSFL
GSPRFHRRKM~~Q~~VPTAEEMSSLTPESSPELAKRSWFGNFISLDKEEQIFL
VLKDKPLSSIKADIVHAFLSIPSLSHSVLSQTSFRAEYKASGGPSV~~F~~QK
PVR~~F~~QVDISSSEGPEPSRRD~~G~~SGGGGIYSVTFTLISG~~P~~SRRFKRVVET
IQAQLLSTHDQPSVQALADEKNGAQTRPAGAPPRSLQPPGPRDPELSS
SPRRGPPKDKLLATNGTLP

Native sequence Amino acids S2 – P778 (end) of human BRSK1.
Residue S29 of the fusion protein is equivalent to S2 of the native
enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1 sites of pFastBAC HTb

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Nucleotide
Sequence of insert

ggatccTCGTCCGGGGCCAAGGAGGGAGGTGGGGGCTCTCCCGCCTACC
ACCTCCCCCACCACCACCACCACCACCAGCACGCCAATATGTGGG
CCCCTATCGGCTGGAGAAGACGCTGGGCAAAGGACAGACAGGGCTGGTT
AAACTCGGGGTCCACTGCATCACGGGTGAGAAGGTCGCCATCAAGATCG
TGAACCGGGAGAAGCTGTCGGAGTCCGGTGTGATGAAGGTGGAGCGGGA
GATCGCCATCCTGAAGCTCATCGAACACCACATGTCTCAAGCTCCAC
GACGTCTACGAGAACAAGAAATATTTGTACCTGGTTCTGGAGCACGTCT
CGGGGGGTGAGCTATTGACTACCTGGTAAAGAAGGGGAGACTGACGCC
CAAGGAGGCCCGAAAGTTCTTCCGCCAGATTGTGTCTGCGCTGGACTTC
TGCCACAGCTACTCCATCTGCCACAGAGACCTAAAGCCCGAGAACCTGC
TTTTGGATGAGAAAAACAACATCCGCATTGCAGACTTCGGCATGGCGTC
CCTGCAGGTGGGGGACAGCCTCCTGGAGACCAGCTGCGGGTCCCCCAT
TATGCGTGTCCAGAGGTGATTAAGGGGAAAAATATGATGGCCGCCGGG
CAGACATGTGGAGCTGTGGAGTCATCCTCTTCGCCCTGCTCGTGGGGC
TCTGCCCTTTGATGACGACAACCTCCGCCAGCTGCTGGAGAAGGTGAAA
CGGGGCGTCTTCCACATGCCCCACTTCATTCCCTCCAGATTGCCAGAGCC
TCCTGAGGGGAATGATCGAAGTGGAGCCCGAAAAAAGGCTCAGTCTGGA
GCAAATTCAGAAACATCCTTGGTACCTAGGCGGGAAACACGAGCCAGAC
CCGTGCCTGGAGCCAGCCCCTGGCCGCCGGGTAGCCATGCGGAGCCTGC
CATCCAACGGAGAGCTGGACCCCGACGTCCTAGAGAGCATGGCATCACT
GGGCTGCTTCAGGGACCGCGAGAGGCTGCATCGCGAGCTGCGCAGTGAG
GAGGAGAACCAAGAAAAGATGATATATTATCTGCTTTTGGATCGGAAGG
AGCGGTATCCCAGCTGTGAGGACCAGGACCTGCCTCCCCGGAATGATGT
TGACCCCCCCCCGGAAGCGTGTGGATTCTCCCATGCTGAGCCGTCACGGG
AAGCGGCGACCAGAGCGGAAGTCCATGGAAGTCTTGAGCATCACCGATG
CCGGGGGTGGTGGCTCCCCTGTACCCACCCGACGGGCTTGGAGATGGC
CCAGCACAGCCAGAGATCCCGTAGCGTCAGTGGAGCCTCCACGGGTCTG
TCCTCCAGCCCTCTAAGCAGCCCAAGGAGTCCGGTCTTTTCTTTTTCAC
CGGAGCCGGGGGCTGGAGATGAGGCTCGAGGCGGGGGCTCCCCGACTTC
CAAACCGCAGACGCTGCCTTCTCGGGGCCCCAGGGGTGGGGGCGCCGGG
GAGCAGCCCCCGCCCCCAGTGCCCGCTCCACACCCTGCCCGCCCCC
CAGGCTCCCCGCGTCTCTGGCGGGACCCCTTGCACTCGCCTCTGCA
CACGCCCCGGGCCAGTCCCACCGGGACCCCGGGGACAACACCACCCCC
AGCCCCGGCGGTGGCGTCCGGGGAGCCGCTGGAGGAGTCTGCTCAACT
CCATCCGCAACAGCTTCCCTGGGCTCCCCTCGCTTTCACCGGCGAAGAT
GCAGGTCCCTACCGCTGAGGAGATGTCCAGCTTGACGCCAGAGTCCCTCC
CCGGAGCTGGCAAAACGCTCCTGGTTCCGGAACTTCATCTCCTTGGACA
AAGAAGAACAATATTCCTCGTGCTAAAGGACAAACCTCTCAGCAGCAT
CAAAGCAGACATCGTCCATGCCTTTCTGTGATCCCCAGCCTGAGTCAC
AGTGTGCTGTACAGACCAGCTTCAGGGCCGAGTACAAGGCCAGTGGCG
GCCCCTCCGTCTTCCAAAAGCCCGTCCGCTTCCAGGTGGACATCAGCTC
CTCTGAGGGTCCAGAGCCCTCCCCGCGACGGGACGGCAGCGGAGGTGGT
GGCATCTACTCCGTACCTTCACTCTCATCTCGGGTCCCAGCCGTCGGT
TCAAGCGAGTGGTGGAGACCATCCAGGCACAGCTCCTGAGCACTCATGA
CCAGCCCTCCGTGCAGGCCCTGGCAGACGAGAAGAACGGGGCCAGACC
CGGCCTGCTGGTGGCCCAACCCGAAGCCTGCAGCCCCCACCAGCCGCC
CAGACCCAGAGCTGAGCAGCTCTCCCCGCCGAGGCCCCCCCAAGGACAA

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GAAGTCCTGGCCACCAACGGGACCCCTCTGCCctgagaattc