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## Standard Operating Procedure

### Preparation of active BRSK2 [2 - 674]

<b><u>Enzyme description:-</u></b>	BRSK2 [2 - 674]
<b><u>Clone number:-</u></b>	DU 1280
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6) tag
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	2 mg/L
<b><u>Calculated molecular mass:-</u></b>	78, 340 daltons
<b><u>Purity:-</u></b>	90 %
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	
	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.
<b><u>Storage temperature:-</u></b>	-70 °C [Long term stability to be determined]
<b><u>Assay:-</u></b>	Standard filter binding assay
<b><u>Assay Buffer:-</u></b>	
	50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc
<b><u>Substrate:-</u></b>	
	Generic peptide [KKLNRTL SFAEPG]      Final concentration: 300 μM
<b><u>Specific activity range:-</u></b>	To be determined

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## Clone Data Sheet - BRSK2 [2 - 674]

**Protein** BRSK2 [2 – 674]

**Clone Number** DU 1280

**Species** Human

**Accession number** AF533878

**Tags** N-terminal His(6)

**Baculovirus expressed protein**

MSYYHHHHHHHDYDIPTTENLYFQGGAMGSTSTGKDGGGAQHAQYVGPYRLE  
KTLGKGQTGLVKLVGVCVTCQKVAIKIVNREKLSVLMKVEREIAILK  
LIEHPHVLKLHDVYENKKYLYLVLEHVS GGELFDYLVKKGRLTPKEARK  
FFRQIISALDFCHSHSICHRDLKPENLLLDEKNNIRIADFGMASLQVGD  
SLEETSCGSPHYACPEVIRGEKYDGRKADVWSCGVILFALLVGALPFDD  
DNLRQLLEKVKRGVFMHPHFIPDCQSLLRGMI EVDAARRLTLEHIQKH  
IWIIGGKNEPEPEQIPRKVQIRSLPSLEDIDPDVLD SMHSLGCFRDRN  
KLLQDLLSEEENQEKMIYFLLDRKERYPSQEDLDLPPRNEIDPPRKR  
VDSPLNRHGKRRPERKSMEVLSVTDGGSPVPARRAIEMAQHGRSRSIS  
GASSGLSTSPLSSPRVTPHSPRGSPLPTPKGTPVHTPKESPAGTPNPT  
PPSSPSVGGVPWRARLNSIKNSFLGSPRFHRRKLQVPTPEEMSNLTPES  
SPELAKKSWFGNFISLEKEEQIFVVIKDKPLSSIKADIVHAFSLIPSL  
SHVISQTSFRAEYKATGGPAVFQKPVKFQVDITYTEGGEAQKENG IYSV  
TFTLLSGPSRRFKRVVETIQAQLLSTHDPPAAQHLSEPPPPAPGLSWGA  
GLKGQKVATSYESSL

**Native sequence** Amino acids S2 – L674 (end) of human BRSK2.  
Residue T29 of fusion protein is equivalent to T2 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 site of pFastBAC HTb

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**Nucleotide  
sequence of  
insert**

ggatccACATCGACGGGGAAGGACGGCGGCGCGCAGCACGCGCAGTATG  
TTGGGCCCTACCGGCTGGAGAAGACGCTGGGCAAGGGGCAGACAGGTCT  
GGTGAAGCTGGGGGTTCACTGCGTCACCTGCCAGAAGGTGGCCATCAAG  
ATCGTCAACCGTGAGAAGCTCAGCGAGTCGGTGCTGATGAAGGTGGAGC  
GGGAGATCGCGATCCTGAAGCTCATTGAGCACCCACGTCCTAAAGCT  
GCACGACGTTTATGAAAACAAAAATATTTGTACCTGGTGCTAGAACAC  
GTGTCAGGTGGTGAGCTCTTCGACTACCTGGTGAAGAAGGGGAGGCTGA  
CGCCTAAGGAGGCTCGGAAGTTCTTCGGCAGATCATCTCTGCGCTGGA  
CTTCTGCCACAGCCACTCCATATGCCACAGGGATCTGAAACCTGAAAAC  
CTCCTGCTGGACGAGAAGAACAACATCCGCATCGCAGACTTTGGCATGG  
CGTCCCTGCAGGTTGGCGACAGCCTGTTGGAGACCAGCTGTGGGTCCCC  
CCACTACGCTGCCCCGAGGTGATCCGGGGGGAGAAGTATGACGGCCGG  
AAGGCGGACGTGTGGAGCTGCGGCGTCATCCTGTTCCCTTGCTGGTGG  
GGGCTCTGCCCTTCGACGATGACAACCTTGCACAGCTGCTGGAGAAGGT  
GAAGCGGGGCGTGTTCACATGCCGCACTTTATCCCGCCGACTGCCAG  
AGTCTGCTACGGGGCATGATCGAGGTGGACGCCGCACGCCGCTCACGC  
TAGAGCACATTCAGAAACACATATGGTATATAGGGGGCAAGAATGAGCC  
CGAACCAGAGCAGCCGATTCCTCGCAAGGTGCAGATCCGCTCGCTGCC  
AGCCTGGAGGACATCGACCCCGACGTGCTGGACAGCATGCACTCACTGG  
GCTGCTTCCGAGACCGCAACAAGCTGCTGCAGGACCTGCTGTCCGAGGA  
GGAGAACCAGGAGAAGATGATTTACTTCCTCCTCCTGGACCGGAAAGAA  
AGGTACCCGAGCCAGGAGGATGAGGACCTGCCCCCCCGAACGAGATAG  
ACCTCCCCGGAAGCGTGTGGACTCCCCGATGCTGAACCGGCACGGCAA  
GCGGCGGCCAGAACGCAAATCCATGGAGGTGCTCAGCGTGACGGACGGC  
GGCTCCCCGGTGCTGCGCGGCGGGCCATTGAGATGGCCACGACGGCC  
AGAGGTCTCGGTCCATCAGCGGTGCCTCCTCAGGCCTTTCACCAGCCC  
ACTCAGCAGCCCCGGGTGACCCCTCACCCCTACCAAGGGGCAGTCCC  
CTCCCCACCCCAAGGGGACACCTGTCCACACGCCAAAGGAGAGCCCGG  
CTGGCACGCCCAACCCACGCCCCGTCCAGCCCAGCGTCGGAGGGGT  
GCCCTGGAGGGCGCGGCTCAACTCCATCAAGAACAGCTTTCTGGGCTCA  
CCCCGCTTCCACCGCCGGAACCTGCAAGTTCCGACGCCGGAGGAGATGT  
CCAACCTGACACCAGAGTCGTCCCCAGAGCTGGCGAAGAAGTCCTGGTT  
TGGGAACCTCATCAGCCTGGAGAAGGAGGAGCAGATCTTCGTGGTCATC  
AAAGACAAACCTCTGAGCTCCATCAAGGTGACATCGTGACGCCTTCC  
TGTCGATTCCCAGTCTCAGCCACAGCGTCATCTCCAAACGAGCTTCCG  
GGCCGAGTACAAGGCCACGGGGGGCCAGCCGTGTTCCAGAAGCCGGTC  
AAGTTCAGGTTGATATCACCTACACGGAGGGTGGGGAGGCGCAGAAGG  
AGAACGGCATCTACTCCGTACCTTACCCTGCTCTCAGGCCCCAGCCG  
TCGCTTCAAGAGGGTGGTGGAGACCATCCAGGCCAGCTGCTGAGCACA  
CACGACCCGCCTGCGGCCAGCACTTGTGAGAACCCCCCCCCACCAGCGC  
CAGGACTAAGCTGGGGTGCTGGGCTTAAGGGCCAGAAGGTGGCCACCAG  
CTACGAGAGTAGCCTCtgaggatcc