Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active BRSK 1 [2 - 778]

Enzyme description:-	BRSK1 [2 - 778]
Clone number:-	DU 1270
Source:-	Recombinant
Expression system:-	Baculovirus expression vector system
Tag:-	N-terminal His(6)
Purification method:-	Ni^{2+}-NTA agarose
Expression level:-	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 [Long term stability to be determined]
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:-
CHKtide [KKKVSRSGLYRSPSMPENLNRPR] Final concentration: 300 μM

Specific activity range:- To be determined

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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	MSYYHHHHHHDYDIPTTENLYFQGAMGS**SSGAKEGGGGSPAYHLPHPHPHPPQHAQYVGPYRLEKTLGKGQTGLVKLGVHCITGQKVAIKIVNREKLSESVLMKVEREIAILKLIEHPHVLKLHDVYENKKYLYLVLEHVSGGELFDYLVKKGRLTPKEARKFFRQIVSALDFCHSYSICHRDLKPENLLLDEKNNIRIADFGMASLQVGDSLLETSCGSPHYACPEVIKGEKYDGRRADMWSCGVILFALLVGALPFDDDNLRQLLEKVKRGVFHMPHFIPPDCQSLLRGMIEVEPEKRLSLEQIQKHPWYLGGKHEPDPCLEPAPGRRVAMRSLPSNGELDPDVLESMASLGCFRDRERLHRELRSEEENQEKMIYYLLLDRKERYPSCEDQDLPPRNDVDPPRKRVDSPMLSRHGKRRPERKSMEVLSITDAGGGGSPVPTRRALEMAQHSQRSRSVSGASTGLSSSPLSSPRSPVFSFSPEPGAGDEARGGGSPTSKTQTLPSRGPRGGGAGEQPPPPSARSTPLPGPPGSPRSSGGTPLHSPLHTPRASPTGTPGTTPPPSPGGGVGGAAWRSRLNSIRNSFLGSPRFHRRKMQVPTAEEMSSLTPESSPELAKRSWFGNFISLDKEEQIFLVLKDKPLSSIKADIVHAFLSIPSLSHSVLSQTSFRAEYKASGGPSVFQKPVRFQVDISSSEGPEPSPRRDGSGGGGIYSVTFTLISGPSRRFKRVVETIQAQLLSTHDQPSVQALADEKNGAQTRPAGAPPRSLQPPPGRPDPELSSSPRRGPPKDKKLLATNGTPLP**
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

Nucleotide Sequence of insert

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ggatccTCGTCCGGGGCCAAGGAGGGAGGTGGGGGCTCTCCCGCCTACC
ACCTCCCCCACCCCCACCCCCACCCACCCCAGCACGCCCAATATGTGGG
CCCCTATCGGCTGGAGAAGACGCTGGGCAAAGGACAGACAGGGCTGGTT
AAACTCGGGGTCCACTGCATCACGGGTCAGAAGGTCGCCATCAAGATCG
TGAACCGGGAGAAGCTGTCGGAGTCGGTGCTGATGAAGGTGGAGCGGGA
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CGGGGGGTGAGCTATTCGACTACCTGGTAAAGAAGGGGAGACTGACGCC
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