

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active BRK [1 - 451]

<u>Enzyme description:-</u>	BRK [1 – 451]
<u>Clone number:-</u>	DU 5916
<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	55, 170.60 daltons
Average Mass	55, 205.80 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.41
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT, 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, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc, 5 mM MnCl ₂	
<u>Substrate:-</u>	
Poly Glu:Tyr (4:1)	Final concentration: 1 mg/ml

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Clone Data Sheet

BRK [1 - 451]

<u>Protein</u>	BRK [1 – 451]
<u>Clone Number</u>	DU 5916
<u>Species</u>	Human
<u>Accession number</u>	NM_005975
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGSMVSRDQAHLGPKYVGLWDFKS RTDEELSFRAQDVHVARKEEQWWATLLDEAGGAVAQGYVPHNYLAER ETVESEPWFPGCISRSEAVRRLQAEGNATGAFLIRVSEKPSADYVLSVR DTQAVRHYKIWRRAGGRLHLNEAVSFLSLPELVNYHRAQSLSHGLRLAA PCRKHEPEPLPHWDDWERPREEFTLCRKLGSQYFGEVFEGLWKDRVQVA IKVISRDNLLHQQMLQSEIQAMKKLRHKKHILALYAVVSVGDPVYIITEL MAKGSLLLELLRDSDEKVLPSSELLDIAWQVAEGMCYLESQNYIHRDLAA RNILVGENTLCKVGDFGLARLIKEDVYLSHDHNIPIKWTAPPEALSRGHY STKSDVWSFGILLHEMFSGQVPYPGMSNHEAFLRVDAGYRMPCPLECP PSVHKLMLTCWCRDPEQRPCFKALRERLSSFTSYENPT
<u>Native sequence</u>	Amino acids M1 – T451 (end) of human BRK. Residue M29 of fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I site of pFastBAC HTb

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Nucleotide sequence of insert

ggatccATGGTGTCCCGGGACCAGGCTCACCTGGGCCCCAAG
TATGTGGGCCTCTGGGACTTCAAGTCCCGGACGGACGAGGAG
CTGAGCTTCCGCGCGGGGGACGTCTTCCACGTGGCCAGGAAG
GAGGAGCAGTGGTGGTGGGCCACGCTGCTGGACGAGGCGGGT
GGGGCCGTGGCCCAGGGCTATGTGCCCCACAACCTACCTGGCC
GAGAGGGAGACGGTGGAGTCGGAACCGTGGTTCTTTGGCTGC
ATCTCCCGCTCGGAAGCTGTGCGTCGGCTGCAGGCCGAGGGC
AACGCCACGGGCGCCTTCCTGATCAGGGTCAGCGAGAAGCCG
AGTGCCGACTACGTCCTGTTCGGTGCGGGACACGCAGGCTGTG
CGGCACTACAAGATCTGGCGGCGTGCCGGGGGCGGCTGCAC
CTGAACGAGGCGGTGTCCTTCCTCAGCCTGCCCCGAGCTTGTG
AACTACCACAGGGCCCAGAGCCTGTCCACGGCCTGCGGCTG
GCCGCGCCCTGCCGGAAGCACGAGCCTGAGCCCCTGCCCCAT
TGGGATGACTGGGAGAGGCCGAGGGAGGAGTTACGCTCTGC
AGGAAGCTGGGGTCCGGCTACTTTGGGGAGGTCTTCGAGGGG
CTCTGGAAGACCGGGTCCAGGTGGCCATTAAGGTGATTTCT
CGAGACAACCTCCTGCACCAGCAGATGCTGCAGTCGGAGATC
CAGGCCATGAAGAAGCTGCGGCACAAACACATCCTGGCGCTG
TACGCCGTGGTGTCCGTGGGGGACCCCGTGTACATCATCACG
GAGCTCATGGCCAAGGGCAGCCTGCTGGAGCTGCTCCGCGAC
TCTGATGAGAAAGTCCTGCCCCGTTTCGGAGCTGCTGGACATC
GCCTGGCAGGTGGCTGAGGGCATGTGTTACCTGGAGTCGCAG
AATTACATCCACCGGGACCTGGCCGCCAGGAACATCCTCGTC
GGGGAAAACACCCCTCTGCAAAGTTGGGGACTTCGGGTTAGCC
AGGCTTATCAAGGAGGACGTCTACCTCTCCCATGACCACAAT
ATCCCCTACAAGTGGACGGCCCCCTGAAGCGCTCTCCCGAGGC
CATTACTCCACCAAATCCGACGTCTGGTCCTTTGGGATTCTC
CTGCATGAGATGTTTCAGCAGGGGTTCAGGTGCCCTACCCAGGC
ATGTCCAACCATGAGGCCTTCCTGAGGGTGGACGCCGGCTAC
CGCATGCCCTGCCCTCTGGAGTGCCCGCCAGCGTGACAAAG
CTGATGCTGACATGCTGGTGCAGGGACCCCGAGCAGAGACCC
TGCTTCAAGGCCCTGCGGGAGAGGCTCTCCAGCTTCACCAGC
TACGAGAACCCGACCTgagaattcccgggtcgactcgagcgg
ccgc