Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active BRK [1 - 451]

Enzyme description:- BRK [1 – 451]

Clone number:- DU 5916

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Calculated molecular mass:-
Monoisotopic 55, 170.60 daltons
Average Mass 55, 205.80 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.41

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,
10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc, 5 mM $MnCl_2$

Substrate:-
Poly Glu:Tyr (4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

BRK [1 - 451]

Protein	BRK [1 – 451]
Clone Number	DU 5916
Species	Human
Accession number	NM_005975
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MVSRDQAHLGPKYVGLWDFKS RTDEELSFRAGDVFHVARKEEQWWWATLLDEAGGAVAQGYVPHNYLAER ETVESEPWFFGCISRSEAVRRLQAEGNATGAFLIRVSEKPSADYVLSVR DTQAVRHYKIWRRAGGRLHLNEAVSFLSLPELVNYHRAQSLSHGLRLAA PCRKHEPEPLPHWDDWERPREEFTLCRKLGSGYFGEVFEGLWKDRVQVA IKVISRDNLLHQQMLQSEIQAMKKLRHKHILALYAVVSVGDPVYIITEL MAKGSLLELLRDSDEKVLPVSELLDIAWQVAEGMCYLESQNYIHRDLAA RNILVGENTLCKVGDFGLARLIKEDVYLSHDHNIPYKWTAPEALSRGHY STKSDVWSFGILLHEMFSRGQVPYPGMSNHEAFLRVDAGYRMPCPLECP PSVHKLMLTCWCRDPEQRPCFKALRERLSSFTSYENPT**
Native sequence	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.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Not*1 site of pFastBAC HTb

Nucleotide sequence of insert

ggatccATGGTGTCCCGGGACCAGGCTCACCTGGGCCCCAAG
TATGTGGGCCTCTGGGACTTCAAGTCCCGGACGGACGAGGAG
CTGAGCTTCCGCGCGGGGGACGTCTTCCACGTGGCCAGGAAG
GAGGAGCAGTGGTGGTGGGCCACGCTGCTGGACGAGGCGGGT
GGGGCCGTGGCCCAGGGCTATGTGCCCCACAACTACCTGGCC
GAGAGGGAGACGGTGGAGTCGGAACCGTGGTTCTTTGGCTGC
ATCTCCCGCTCGGAAGCTGTGCGTCGGCTGCAGGCCGAGGGC
AACGCCACGGGCGCCTTCCTGATCAGGGTCAGCGAGAAGCCG
AGTGCCGACTACGTCCTGTCGGTGCGGGACACGCAGGCTGTG
CGGCACTACAAGATCTGGCGGCGTGCCGGGGGCCGGCTGCAC
CTGAACGAGGCGGTGTCCTTCCTCAGCCTGCCCGAGCTTGTG
AACTACCACAGGGCCCAGAGCCTGTCCCACGGCCTGCGGCTG
GCCGCGCCCTGCCGGAAGCACGAGCCTGAGCCCCTGCCCCAT
TGGGATGACTGGGAGAGGCCGAGGGAGGAGTTCACGCTCTGC
AGGAAGCTGGGGTCCGGCTACTTTGGGGAGGTCTTCGAGGGG
CTCTGGAAAGACCGGGTCCAGGTGGCCATTAAGGTGATTTCT
CGAGACAACCTCCTGCACCAGCAGATGCTGCAGTCGGAGATC
CAGGCCATGAAGAAGCTGCGGCACAAACACATCCTGGCGCTG
TACGCCGTGGTGTCCGTGGGGGACCCCGTGTACATCATCACG
GAGCTCATGGCCAAGGGCAGCCTGCTGGAGCTGCTCCGCGAC
TCTGATGAGAAAGTCCTGCCCGTTTCGGAGCTGCTGGACATC
GCCTGGCAGGTGGCTGAGGGCATGTGTTACCTGGAGTCGCAG
AATTACATCCACCGGGACCTGGCCGCCAGGAACATCCTCGTC
GGGGAAAACACCCTCTGCAAAGTTGGGGACTTCGGGTTAGCC
AGGCTTATCAAGGAGGACGTCTACCTCTCCCATGACCACAAT
ATCCCCTACAAGTGGACGGCCCCTGAAGCGCTCTCCCGAGGC
CATTACTCCACCAAATCCGACGTCTGGTCCTTTGGGATTCTC
CTGCATGAGATGTTCAGCAGGGGTCAGGTGCCCTACCCAGGC
ATGTCCAACCATGAGGCCTTCCTGAGGGTGGACGCCGGCTAC
CGCATGCCCTGCCCTCTGGAGTGCCCGCCCAGCGTGCACAAG
CTGATGCTGACATGCTGGTGCAGGGACCCCGAGCAGAGACCC
TGCTTCAAGGCCCTGCGGGAGAGGCTCTCCAGCTTCACCAGC
TACGAGAACCCGACCtgagaattcccgggtcgactcgagcgg
ccgc