University of Dundee

Standard Operating Procedure

Preparation of active PAK6 [2 – 681]

Enzyme description:- PAK6 [2 - 681]

Clone number:- DU 4190

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 101, 870.40 daltons
Average Mass 101, 935.12 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.03

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, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
RRRLSFAEPG Final concentration: 300 μM

Specific actiity range:- To be determined

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Clone Data Sheet - PAK6 [2 - 681]

Protein PAK6 [2 - 681]

Clone number DU 4190

Species Human

Accession number Q9NQU5

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**FRKKKKKRPEI**
SAPQNFQHRVHTSFDPKEGKFVGLPPQWQNILDTLRRPKPVVDPSRITR
VQLQPMKTVVRGSAMPVDGYISGLLNDIQKLSVISSNTLRGRSPTSRRR
AQSLGLLGDEHWATDPDMYLQSPQSERTDPHGLYLSCNGGTPAGHKQMP
WPEPQSPRVLPNGLAAKAQSLGPAEFQGASQRCLQLGACLQSSPPGASP
PTGTNRHGMKAAKHGSEEARPQSCLVGSATGRPGGEGSPSPKTRESSLK
RRLFRSMFLSTAATAPPSSSKPGPPPQSKPNSSFRPPQKDNPPSLVAKA
QSLPSDQPVGTFSPLTTSDTSSPQKSLRTAPATGQLPGRSSPAGSPRTW
HAQISTSNLYLPQDPTVAKGALAGEDTGVVTHEQFKAALRMVVDQGDPR
LLLDSYVKIGEGSTGIVCLAREKHSGRQVAVKMMDLRKQQRRELLFNEV
VIMRDYQHFNVVEMYKSYLVGEELWVLMEFLQGGALTDIVSQVRLNEEQ
IATVCEAVLQALAYLHAQGVIHRDIKSDSILLTLDGRVKLSDFGFCAQI
SKDVPKRKSLVGTPYWMAPEVISRSLYATEVDIWSLGIMVIEMVDGEPP
YFSDSPVQAMKRLRDSPPPKLKNSHKVSPVLRDFLERMLVRDPQERATA
QELLDHPFLLQTGLPECLVPLIQLYRKQTSTC

Native sequence Amino acids F2 – C681 (end) of human PAK6.
Residue F235 of the fusion protein is equivalent to F2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Eco*R1 and *Not*1site of pGEX 6P-1

Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
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GGGATCCCCGGAATTCTTCCGCAAGAAAAAGAAGAAACGCCCTGAGATC
TCAGCGCCACAGAACTTCCAGCACCGTGTCCACACCTCCTTCGACCCCA
AAGAAGGCAAGTTTGTGGGCCTCCCCCCACAATGGCAGAACATCCTGGA
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GTGCAGCTCCAGCCCATGAAGACAGTGGTGCGGGGCAGCGCGATGCCTG
TGGATGGCTACATCTCGGGGCTGCTCAACGACATCCAGAAGTTGTCAGT
CATCAGCTCCAACACCCTGCGTGGCCGCAGCCCCACCAGCCGGCGGCGG
GCACAGTCCCTGGGGCTGCTGGGGGATGAGCACTGGGCCACCGACCCAG
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CTACCTCAGCTGCAACGGGGGCACACCAGCAGGCCACAAGCAGATGCCG
TGGCCCGAGCCACAGAGCCCACGGGTCCTGCCCAATGGGCTGGCTGCAA
AGGCACAGTCCCTGGGCCCCGCCGAGTTTCAGGGTGCCTCGCAGCGCTG
TCTGCAGCTGGGTGCCTGCCTGCAGAGCTCCCCACCAGGAGCCTCGCCC
CCCACGGGCACCAATAGGCATGGAATGAAGGCTGCCAAGCATGGCTCTG
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AGGTGGGGAAGGCAGCCCTAGCCCTAAGACCCGGGAGAGCAGCCTGAAG
CGCAGGCTATTCCGAAGCATGTTCCTGTCCACTGCTGCCACAGCCCCTC
CAAGCAGCAGCAAGCCAGGCCCTCCACCACAGAGCAAGCCCAACTCCTC
TTTCCGACCGCCGCAGAAAGACAACCCCCCAAGCCTGGTGGCCAAGGCC
CAGTCCTTGCCCTCGGACCAGCCGGTGGGGACCTTCAGCCCTCTGACCA
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GCAGTTCAAGGCTGCGCTCAGGATGGTGGTGGACCAGGGTGACCCCCGG
CTGCTGCTGGACAGCTACGTGAAGATTGGCGAGGGCTCCACCGGCATCG
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GTGATCATGCGGGACTACCAGCACTTCAACGTGGTGGAGATGTACAAGA
GCTACCTGGTGGGCGAGGAGCTGTGGGTGCTCATGGAGTTCCTGCAGGG
AGGAGCCCTCACAGACATCGTCTCCCAAGTCAGGCTGAATGAGGAGCAG
ATTGCCACTGTGTGTGAGGCTGTGCTGCAGGCCCTGGCCTACCTGCATG
CTCAGGGTGTCATCCACCGGGACATCAAGAGTGACTCCATCCTGCTGAC

CCTCGATGGCAGGGTGAAGCTCTCGGACTTCGGATTCTGTGCTCAGATC
AGCAAAGACGTCCCTAAGAGGAAGTCCCTGGTGGGAACCCCCTACTGGA
TGGCTCCTGAAGTGATCTCCAGGTCTTTGTATGCCACTGAGGTGGATAT
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TACTTCAGTGACTCCCCAGTGCAAGCCATGAAGAGGCTCCGGGACAGCC
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CTTCCTGGAGCGGATGCTGGTGCGGGACCCCCAAGAGAGAGCCACAGCC
CAGGAGCTCCTAGACCACCCCTTCCTGCTGCAGACAGGGCTACCTGAGT
GCCTGGTGCCCCTGATCCAGCTCTACCGAAAGCAGACCTCCACCTGCtg
agcggccgc