University of Dundee

Standard Operating Procedure

Preparation of active PAK5 (PAK7) [2 – 719]

Enzyme description:- PAK5 (PAK7) [2 - 719]

Clone number:- DU 4188

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 107, 742.71 daltons
Average Mass 107, 811.26 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.92

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 - PAK5 (PAK7) [2 - 719]

Protein PAK5 [2 - 719]

Clone number DU 4188

Species Human

Accession number Q9P286

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**FGKKKKKIEIS**
GPSNFEHRVHTGFDPQEQKFTGLPQQWHSLLADTANRPKPMVDPSCITP
IQLAPMKTIVRGNKPCKETSINGLLEDFDNISVTRSNSLRKESPPTPDQ
GASSHGPGHAEENGFITFSQYSSESDTTADYTTEKYREKSLYGDDLDPY
YRGSHAAKQNGHVMKMKHGEAYYSEVKPLKSDFARFSADYHSHLDSLSK
PSEYSDLKWEYQRASSSSPLDYSFQFTPSRTAGTSGCSKESLAYSESEW
GPSLDDYDRRPKSSYLNQTSPQPTMRQRSRSGSGLQEPMMPFGASAFKT
HPQGHSYNSYTYPRLSEPTMCIPKVDYDRAQMVLSPPLSGSDTYPRGPA
KLPQSQSKSGYSSSSHQYPSGYHKATLYHHPSLQSSSQYISTASYLSSL
SLSSSTYPPPSWGSSSDQQPSRVSHEQFRAALQLVVSPGDPREYLANFI
KIGEGSTGIVCIATEKHTGKQVAVKKMDLRKQQRRELLFNEVVIMRDYH
HDNVVDMYSSYLVGDELWVVMEFLEGGALTDIVTHTRMNEEQIATVCLS
VLRALSYLHNQGVIHRDIKSDSILLTSDGRIKLSDFGFCAQVSKEVPKR
KSLVGTPYWMAPEVISRLPYGTEVDIWSLGIMVIEMIDGEPPYFNEPPL
QAMRRIRDSLPPRVKDLHKVSSVLRGFLDLMLVREPSQRATAQELLGHP
FLKLAGPPSCIVPLMRQYRHH

Native sequence Amino acids F2 – H719 (end) of human PAK5 (PAK7).
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
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCT
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GGCCCGTCCAACTTTGAACACAGGGTTCATACTGGGTTTGATCCACAAG
AGCAGAAGTTTACCGGCCTTCCCCAGCAGTGGCACAGCCTGTTAGCAGA
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CCTTCTCCCAGTATTCCAGCGAATCCGATACTACTGCTGACTACACGAC
CGAAAAGTACAGGGAGAAGAGTCTCTATGGAGATGATCTGGATCCGTAT
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AGCACGGGGAGGCCTACTATTCTGAGGTGAAGCCTTTGAAATCCGATTT
TGCCAGATTTTCTGCCGATTATCACTCACATTTGGACTCACTGAGCAAA
CCAAGTGAATACAGTGACCTCAAGTGGGAGTATCAGAGAGCCTCGAGTA
GCTCCCCTCTGGATTATTCATTCCAATTCACACCTTCTAGAACTGCAGG
GACCAGCGGGTGCTCCAAGGAGAGCCTGGCGTACAGTGAAAGTGAATGG
GGACCCAGCCTGGATGACTATGACAGGAGGCCAAAGTCTTCGTACCTGA
ATCAGACAAGCCCTCAGCCCACCATGCGGCAGAGGTCCAGGTCAGGCTC
GGGACTCCAGGAACCGATGATGCCATTTGGAGCAAGTGCATTTAAAACC
CATCCCCAAGGACACTCCTACAACTCCTACACCTACCCTCGCTTGTCCG
AGCCCACAATGTGCATTCCAAAGGTGGATTACGATCGAGCACAGATGGT
CCTCAGCCCTCCACTGTCAGGGTCTGACACCTACCCCAGGGGCCCTGCC
AAACTACCTCAAAGTCAAAGCAAATCGGGCTATTCCTCAAGCAGTCACC
AGTACCCGTCTGGGTACCACAAAGCCACCTTGTACCATCACCCCTCCCT
GCAGAGCAGTTCGCAGTACATCTCCACGGCTTCCTACCTGAGCTCCCTC
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GCTGGTGGTCAGCCCAGGAGACCCCAGGGAATACTTGGCCAACTTTATC
AAAATCGGGGAAGGCTCAACCGGCATCGTATGCATCGCCACCGAGAAAC
ACACAGGGAAACAAGTTGCAGTGAAGAAAATGGACCTCCGGAAGCAACA
GAGACGAGAACTGCTTTTCAATGAGGTCGTGATCATGCGGGATTACCAC
CATGACAATGTGGTTGACATGTACAGCAGCTACCTTGTCGGCGATGAGC
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GACTCACACCAGAATGAATGAAGAACAGATAGCTACTGTCTGCCTGTCA
GTTCTGAGAGCTCTCTCCTACCTTCATAACCAAGGAGTGATTCACAGGG
ACATAAAAAGTGACTCCATCCTCCTGACAAGCGATGGCCGGATAAAGTT
GTCTGATTTTGGTTTCTGTGCTCAAGTTTCCAAAGAGGTGCCGAAGAGG
AAATCATTGGTTGGCACTCCCTACTGGATGGCCCCTGAGGTGATTTCTA
GGCTACCTTATGGGACAGAGGTGGACATCTGGTCCCTCGGGATCATGGT
GATAGAAATGATTGATGGCGAGCCCCCCTACTTCAATGAGCCTCCCCTC
CAGGCGATGCGGAGGATCCGGGACAGTTTACCTCCAAGAGTGAAGGACC
TACACAAGGTTTCTTCAGTGCTCCGGGGATTCCTAGACTTGATGTTGGT
GAGGGAGCCCTCTCAGAGAGCAACAGCCCAGGAACTCCTCGGACATCCA
TTCTTAAAACTAGCAGGTCCACCGTCTTGCATTGTCCCCCTCATGAGAC
AATACAGGCATCACtgagcggccgc