

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active PAK5 [2 – 719]**

**Enzyme description:-** PAK5 [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  $\mu$ M

**Specific activity range:-** To be determined

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**Clone Data Sheet**

**PAK5 [2 - 719]**

**Protein** PAK5 [2 - 719]

**Clone number** DU 4188

**Species** Human

**Accession number** Q9P286

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPEFFGKKKKKIEIS  
**GPSNFEHRVHTGFDPOEQKFTGLPQQWHSLLADTANRPKPMVDPSCITP**  
**IQLAPMKTIVRGNKPKETSINGLLEDFDNISVTRSNSLRKESPTPDQ**  
**GASSHGPGHAEENGFITFSQYSSSDTTADYTTEKYREKSLYGDDLDPY**  
**YRGSHAAKQNGHVMKMKHGEAYYSEVKPLKSDFARFSADYHSHLDSLK**  
**PSEYSDLKWEYQRASSSSPLDYSFQFTPSRTAGTSGCSKESLAYSESEW**  
**GPSLDDYDRRPKSSYLNQTSPOPTMRQRSRSGSGLQEPMPFGASAFKT**  
**HPQHSYNSYTYPRLSEPTMCIPKVDYDRAQMVLSPLSGSDTYPRGPA**  
**KLPQSQSKSGYSSSSHQYPSGYHKATLYHHPQLQSSSQYISTASYLSSL**  
**SLSSSTYPPPSWGSSSDQPSRVSHQFRAALQLVSPGDPREYLANFI**  
**KIGEGSTGIVCIATEKHTGKQVAVKKMDLRKQORRELLFNEVVIMRDYH**  
**HDNVVDMYSSYLVGDELWVMEFLEGGALTDIVTHTRMNEEQIATVCLS**  
**VLRALSYLHNQGV IHRDIKSDSILLTSDGRIKLSDFGFCQVSKVPPKR**  
**KSLVGTPTYWMAPEVISRLPYGTEVDIWSLGMVIMIDGEPYPFNEPPL**  
**QAMRRIRDSLPPRVKDLHKVSSVLRGFLDLMLVREPSQRATAQELLGHP**  
**FLKLAGPPSCIVPLMRQYRHH**

**Native sequence** Amino acids F2 – H719 (end) of human PAK5.  
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*I site of pGEX 6P-1

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### Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA  
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTA  
TGAGCGGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT  
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA  
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT  
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG  
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT  
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA  
AATGTTTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT  
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT  
ACATGGACCCAATGTGCCTGGATGCGTTCCAAAATTAGTTTGTTTTTAA  
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC  
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG  
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCT  
GGGATCCCCGGAATTCTTTGGGAAGAAAAAGAAAAGATTGAAATATCT  
GGCCCGTCCAACCTTGAACACAGGGTTCATACTGGGTTTGATCCACAAG  
AGCAGAAGTTTACCGGCCTTCCCAGCAGTGGCACAGCCTGTTAGCAGA  
TACGGCCAACAGGCCAAAGCCTATGGTGGACCCTTCATGCATCACACC  
ATCCAGCTGGCTCCTATGAAGACAATCGTTAGAGGAAACAAACCCTGCA  
AGGAAACCTCCATCAACGGCCTGCTAGAGGATTTTGACAACATCTCGGT  
GACTCGCTCCAACCTCCCTAAGGAAAGAAAGCCACCCACCCAGATCAG  
GGAGCCTCCAGCCACGGTCCAGGCCACGCGGAAGAAAATGGCTTCATCA  
CCTTCTCCAGTATTCCAGCGAATCCGATACTACTGCTGACTACACGAC  
CGAAAAGTACAGGGAGAAGAGTCTCTATGGAGATGATCTGGATCCGTAT  
TATAGAGGCAGCCACGCAGCCAAGCAAAATGGGCACGTAATGAAAATGA  
AGCACGGGGAGGCCTACTATTCTGAGGTGAAGCCTTTGAAATCCGATTT  
TGCCAGATTTTCTGCCGATTATCACTCACATTTGGACTCACTGAGCAAA  
CCAAGTGAATACAGTGACCTCAAGTGGGAGTATCAGAGAGCCTCGAGTA  
GCTCCCCCTGATTATTCAATCCAATTCACACCTTCTAGAACTGCAGG  
GACCAGCGGGTGCTCCAAGGAGAGCCTGGCGTACAGTGAAAGTGAATGG  
GGACCCAGCCTGGATGACTATGACAGGAGGCCAAAGTCTTCGTACCTGA  
ATCAGACAAGCCCTCAGCCCACCATGCGGCAGAGGTCCAGGTCAGGCTC  
GGGACTCCAGGAACCGATGATGCCATTTGGAGCAAGTGCATTTAAAACC  
CATCCCCAAGGACACTCCTACAACCTTACACCTACCCCTCGCTTGTCCG  
AGCCACAATGTGCATTCCAAAGGTGGATTACGATCGAGCACAGATGGT  
CCTCAGCCCTCCACTGTCAGGGTCTGACACCTACCCAGGGGCCCTGCC  
AAACTACCTCAAAGTCAAAGCAAATCGGGCTATTCTCAAGCAGTCACC  
AGTACCCGTCTGGGTACCACAAAGCCACCTTGTACCATCACCCCTCCCT  
GCAGAGCAGTTTCGAGTACATCTCCACGGCTTCTACCTGAGCTCCCTC  
AGCCTCTCATCCAGCACCTACCCGCCGCCAGCTGGGGCTCCTCCTCCG  
ACCAGCAGCCCTCCAGGGTGTCCCATGAACAGTTTCGGGCGGCCCTGCA  
GCTGGTGGTCAGCCAGGAGACCCAGGGAATACTTGGCCAACCTTTATC  
AAAATCGGGGAAGGCTCAACCGGCATCGTATGCATCGCCACCGAGAAAC  
ACACAGGGAAACAAGTTGCAGTGAAGAAAATGGACCTCCGGAAGCAACA  
GAGACGAGAAGTGTCTTTCAATGAGGTCGTGATCATGCGGGATTACCAC  
CATGACAATGTGGTTGACATGTACAGCAGCTACCTTGTGCGCGATGAGC  
TCTGGGTGGTCATGGAGTTTCTAGAAGGTGGTGCCTTGACAGACATTTGT

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GACTCACACCAGAATGAATGAAGAACAGATAGCTACTGTCTGCCTGTCA  
GTTCTGAGAGCTCTCTCCTACCTTCATAACCAAGGAGTGATTCACAGGG  
ACATAAAAAGTGACTCCATCCTCCTGACAAGCGATGGCCGGATAAAGTT  
GTCTGATTTTGGTTTCTGTGCTCAAGTTTCCAAAGAGGTGCCGAAGAGG  
AAATCATTGGTTGGCACTCCCTACTGGATGGCCCCTGAGGTGATTTCTA  
GGCTACCTTATGGGACAGAGGTGGACATCTGGTCCCTCGGGATCATGGT  
GATAGAAATGATTGATGGCGAGCCCCCTACTTCAATGAGCCTCCCCTC  
CAGGCGATGCGGAGGATCCGGGACAGTTTACCTCCAAGAGTGAAGGACC  
TACACAAGGTTTCTTCAGTGCTCCGGGGATTCCCTAGACTTGATGTTGGT  
GAGGGAGCCCTCTCAGAGAGCAACAGCCCAGGAACTCCTCGGACATCCA  
TTCTTAAACTAGCAGGTCCACCGTCTTGCATTGTCCCCCTCATGAGAC  
AATACAGGCATCACTgagcggccgc