

Division of Signal Transduction Therapy

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 μ M

Specific activity range:- To be determined

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Clone Data Sheet

PAK6 [2 - 681]

<u>Protein</u>	PAK6 [2 - 681]
<u>Clone number</u>	DU 4190
<u>Species</u>	Human
<u>Accession number</u>	Q9NQU5
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPEFFFRKKKKKRPEI SAPQNFQHRVHTSFDPKEGKFVGLPPQWQNILDTLRRPKPVVDPSRITR VQLQPMKTVVRGSAMPVDGYISGLLNDIQKLSVISSNTLRGRSPTSRRR AQSLGLLGDEHWATDPDMYLQSPQSERTDPHGLYLSCNGGTPAGHKQMP WPEPQSPRVL PNGLAAKAQSLGPAEFQGASQRCLOLGACLOSSPPGASP PTGTNRHGMKAAKHGSEEARPQSCLVGSATGRPGGEGSPSPKTRESSLK RRLFRSMFLSTAATAPPSSSKPGPPPQSKPNSSFRPPQKDNPPSLVAKA QSLPSDQPVGTFSPLTTSSTSSPQKSLRTAPATGQLPGRSSPAGSPRTW HAQISTSNLYLPQDPTVAKGALAGEDTGVTTHEQFKAALRMVVDQGDPR LLLD SYVKIGEGSTGIVCLAREKHSGRQVAVKMMDLRKQQRRELLFNEV VIMRDYQHFNVVEMYKSYLVGEELWVLMEFLQGGALTDIVSQVRLNEEQ IATVCEAVLQALAYLHAQGV IHRDIKSDSILLTLDGRVKLSDFGFC AQI SKDVPKRKSLVGTPYWMAPEVISRSLYATEVDIWSLGIMVIEMVDGEPP YFSDSPVQAMKRLRDSPPPKLKNSHKVSPVLRDFLERMLVRDPQERATA QELLDHPFLLQTGLPECLVPLIQLYRKQTSTC</p>
<u>Native sequence</u>	<p>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.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Eco</i> R1 and <i>Not</i> I site of pGEX 6P-1

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Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
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GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
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CCTCGATGGCAGGGTGAAGCTCTCGGACTTCGGATTCTGTGCTCAGATC
AGCAAAGACGTCCCTAAGAGGAAGTCCCTGGTGGGAACCCCTACTGGA
TGGCTCCTGAAGTGATCTCCAGGTCTTTGTATGCCACTGAGGTGGATAT
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