University of Dundee

Standard Operating Procedure

Preparation of active PAK4 [2 – 591]

Enzyme description:- PAK4 [2 - 591]

Clone number:- DU 4186

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 6 mg/L

Calculated molecular mass:-
Monoisotopic 90, 707.78 daltons
Average Mass 90, 765.02 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.09

Purity:- 85 %

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 activity range:- To be determined

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Clone Data Sheet - PAK4 [2 - 591]

Protein	PAK4 [2 - 591]
Clone number	DU 4186
Species	Human
Accession number	O96013
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**FGKRKKRVEISAPS** **NFEHRVHTGFDQHEQKFTGLPRQWQSLIEESARRPKPLVDPACITSIQP** **GAPKTIVRGSKGAKDGALTLLLDEFENMSVTRSNSLRRDSPPPPARARQ** **ENGMPEEPATTARGGPGKAGSRGRFAGHSEAGGGSGDRRRAGPEKRPKS** **SREGSGGPQESSRDKRPLSGPDVGTPQPAGLASGAKLAAGRPFNTYPRA** **DTDHPSRGAQGEPHDVAPNGPSAGGLAIPQSSSSSSRPPTRARGAPSPG** **VLGPHASEPQLAPPACTPAAPAVPGPPGPRSPQREPQRVSHEQFRAALQ** **LVVDPGDPRSYLDNFIKIGEGSTGIVCIATVRSSGKLVAVKKMDLRKQQ** **RRELLFNEVVIMRDYQHENVVEMYNSYLVGDELWVVMEFLEGGALTDIV** **THTRMNEEQIAAVCLAVLQALSVLHAQGVIHRDIKSDSILLTHDGRVKL** **SDFGFCAQVSKEVPRRKSLVGTPYWMAPELISRLPYGPEVDIWSLGIMV** **IEMVDGEPPYFNEPPLKAMKMIRDNLPPRLKNLHKVSPSLKGFLDRLLV** **RDPAQRATAAELLKHPFLAKAGPPASIVPLMRQNRTR**
Native sequence	Amino acids F2 – R591 (end) of human PAK4. Residue F232 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	*Bam*H1 and *Not*1site of pGEX 6P-1

Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
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GCACAAGGTGTCGCCATCCCTGAAGGGCTTCCTGGACCGCCTGCTGGTG
CGAGACCCTGCCCAGCGGGCCACGGCAGCCGAGCTGCTGAAGCACCCAT
TCCTGGCCAAGGCAGGGCCGCCTGCCAGCATCGTGCCCCTCATGCGCCA
GAACCGCACCAGAtgagcggccgc