PAK4

PDB:2J0I

Revision

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Entry Clone Accession:gi|5031975
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SGC Clone Accession:PAK4A-c010
Tag: PreScission(TM) (rhinovirus 3C)- protease cleavable (*) GST tag.
mspilgywkikglvqptrllleyleekyeehlyerdegdkwrnkkfelglefpnlpyyidgdvkltqsmaiiryiadkhnmlggcpke
raeismlegavldirygvsriayskdfetlkvdflsklpemlkmfedrlchktylngdhvthpdfmlydaldvvlymdpmcldafpkl
vcfkkrieaipqidkylkssky iawplqgwqatfgggdhppksdlevlfq*gplgs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

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After cleavage:
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gplgsSPQREPQRVSHEQFRAALQLVVDPGDPRSYLDNFIKIGEGSTGIVCIATVRSSGKLVA
VKKMDLRKQQRRELLFNEVVIMRDYQHENVVEMYNSYLVGDELWVVMEFLEGGALTD
IVTHTRMNEEQIAAVCLAVLQALSVLHAQGVIHRDIKSDSILLTHDGRVKLSDFGFCAQVS
KEVPRRKSLVGTPYWMAPELISRLPYGPEVDIWSLGIMVIEMVDGEPPYFNEPPLKAMK
MIRDNLPPRLKNLHKVSPSLKGFLDRLLVRDPAQRATAAELLKHPFLAKAGPPASIVPLM
RQNRTR
Vector:pGEX-6P2

Growth

Medium:
Antibiotics:
Procedure: Starter cultures from freshly transformed colonies in 10 ml LB, 0.1 mg/ml ampicillin

were grown overnight. This was diluted 1:1000 in fresh media (6L) and was grown at 37°C to an OD600 of 0.3 and than transferred to 18°C. Expression was induced at an OD600 of 0.8 using 1 mM IPTG. Cells were harvested after 12h by centrifugation, transferred to 50-ml tubes, and frozen in liquid nitrogen.

Purification

Procedure
Column 1: Glutathione Sepharose 4B affinity, 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.
Column 2: SEC (S75 or S200)

Supernatant was applied at gravity flow, followed by a wash with 30 ml binding buffer. The GST-fusion was cleaved while bound to the column by addition of PreScission protease. The column was gently rotated overnight a 4°C. The protein was subsequently eluted with 3 bed volumes of binding buffer.

Fractions containing PAK4 were concentrated and applied to a S75 gel fitration column equilibrated in SEC buffer. PAK4 eluted with a retention time corresponding to a monomeric protein of 40 kDa.

Extraction

Procedure
The cell pellets (20 g wet wt) were re-suspended in 50 ml extraction buffer, and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 60,000 rpm.
Concentration: Centricons 10 kDa cut off in same buffer. The mass of the recombinant Pak4 corresponded to the expected mass and one PO4 moiety. The protein was >95% pure as judged by SDS PAGE. ESI-MS spectra showed that the recombinant protein had a molecular weight of 34456- which corresponds to the mono-phosphorylated protein, in agreement with the theoretical mass of 34376 Da.
Ligand
MassSpec:
Crystallization: Pak4 was concentrated to 10 mg/ml. The protein was crystallized is 96 well sitting drop Greiner plates using the vapour diffusion method at 4°C mixing 100 nl protein solution with a well solution containing 0.20M K 3 (cit); 0.1M BTProp pH 6.5; 20.0% PEG 3350; 10.0% EtGly. Crystals were frozen in the crystallization buffer containing 15% ethylene glycole.
NMR Spectroscopy:
Data Collection: Resolution: 1.6Å; X-ray source: SLS-X10
Data Processing: