

PAK5

PDB:2F57

Revision

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Entry Clone Accession:gi|24308191

Entry Clone Source:MGC

SGC Clone Accession:

Tag:mhhhhhssgvdlgtenlyfq*(m)TEV-cleavable (*) N-terminal his6 tag.

Host:BL21 (DE3)

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfq*smSRVSHEQFRAALQLVSPGDPREYLANFIKIGEGSTGIVCIATEKHTGKQVAVKKMDLRKQQR
RELLFNEVVIMRDYHHDNVVDMYSSYLVGDELWVMEFLEGGALTDIVTHTRMNEEQIATVCLSVLRALSYLHNQGV IHRDIKSDSI
LLTSDGRIKLSDFGFCQAQVSKEVPKRKXLVGTPYWMapevisRLPYGTEVDIWSLGIMVIEMIDGEPPYFNEPPLQAMRRIRDSLPP
RVKDLHKVSSVLRGFLDMLVREPSQRATAQELLGHPFLKLAGPPSCIVPLMRQ

Vector:pLIC-SGC1

Growth

Medium:

Antibiotics:

Procedure:1mL from a 10 mL LB overnight culture containing 50 µg/mL kanamycin was used to inoculate 1 liter of LB media containing 50 µg/mL kanamycin. Cultures were grown at 37°C until the OD600 reached ~0.3. After that the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

Purification

Procedure

Column 1: Ni-affinity chromatography.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500mM NaCl, 5% Glycerol. Wash buffer: 50 mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol. Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 50 to 250mM Imidazole, 5% Glycerol.

Procedure: 5 mL of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 30 mL binding buffer. The lysate was applied to the column which was subsequently washed with 3 x 10 mL wash buffer. PAK5 was eluted by a step gradient generated by 5 mL portions of elution buffer with increasing concentration of imidazole (concentrations used: 50mM, 100mM, 250mM). The eluted protein fractions were collected and analyzed by SDS - PAGE . Most of PAK5 eluted at an imidazole concentration of 100 mM. After elution DTT was added to a final concentration of 10mM.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)

SEC -Buffers: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM DTT.

Procedure: The fractions eluted of the Ni-affinity chromatography were pooled and concentrated to about 1 mL using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 1 mL/min. PAK5 eluted at 65 minutes corresponding to a retention time of a monomeric protein of that size. Eluted fractions were 95% pure as judged by SDS - PAGE .

Protein concentration: Centricon with a 10kDa cut off in SEC -buffer

Extraction

Procedure

Cell pellets were lysed using a high pressure cell disrupter. The lysate was centrifuged at 50,000 rpm for 40 minutes and the supernatant collected for purification.

Concentration:

Ligand

MassSpec:

Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/mL by mixing 100nl of the concentrated protein with 100nl of a well solution containing 0.20M Na/KPO₄, 0.1M Bis Tris Propane pH 7.5, 20.0% PEG 3350, 10.0% Etylene glycole at 4°C.

NMR Spectroscopy:

Data Collection:

Data Processing: