MAP3K10

PDB:2RF0

Revision

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Entry Clone Accession:NM_002446 Variant
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SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3) phage resistant Rosetta strain

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smGTTPAGPVWTAVFDYEAAGDEELTLRRGDRVQVLSQDCAVSGDEGWWTGQLPSGRVGVFPSNY
VAP

Vector:pNIC28-Bsa4

Growth

Medium:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycine and 35 µg/ml chloramphenicole was used to inoculate 1 liter of LB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD600 reached ~0.3. After that the temperature was adjusted to 20°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% glycerol, 20 mM imidazole.
Antibiotics:
Procedure:

Purification

Procedure
Column 1: Ni-affinity chromatography.Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 5% Glycero, 20mM Imidazolel. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 30 mM Imidazole , 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 50 to 250 mM 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 50 ml binding buffer. The lysate was applied to the column which was subsequently washed with 50 ml wash buffer 1 and 2. The

protein was eluted by gravity flow by applying 5 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150mM, 250 mM); fractions were collected until essentially all protein was eluted. The eluted protein was analyzed by SDS - PAGE. DTT was added to the protein sample to a final concentration of 10mM.Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)SEC-Buffers: 50 mM Hepes, pH 7.5, 150 mM NaCl, 5 mM DTT.Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 mls 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 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure
Extraction method: Cell pellets were lysed by sonication. The lysate was centrifuged at 19,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration: Centricon with a 10kDa cut off in SEC-buffer.
Ligand
MassSpec: The mass of the protein determined by ESI -MS-tof corresponded to the theoretical mass of Dalton 9724.5 calculated for the protein before TEV cleavage.
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 20 mg/ml by mixing 150nl of the concentrated protein with 50nl of a well solution containing: 0.20M Na(malonate); 0.1M BTProp pH 8.5; 20.0% PEG 3350 and 10.0% EtGly in the presence of 1 mM of the peptide: \"DRERPHVRRRRG\".
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution supplemented with an additional 20% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line SAX10 to 2.0 Å resolution.
Data Processing: