MAP3K5

PDB:2CLQ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|5174547
Entry Clone Source:MGC
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

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Final protein sequence: mhhhhhhssgvdlgtenlyfqsmRSTEEGDCESDLLEYDYEYDENGDRVVLGKGTYGIVYAGR
DLSNQVRIAIKEIPERDSRYSQPLHEEIALHKHLKHKNIVQYLGSFSENGFIKIFMEQVPGGSLSALLRSKWGPLKDNEQTIGFYTK
QILEGLKYLHDNQIVHRDIKGDNVLINTYSGVLKISDFGTSKRLAGINPCTETFTGTLQYMAPEIIDKGPRGYGKAADIWSLGCTII
EMATGKPPFYELGEPQAAMFKVGMFKVHPEIPESMSAEAKAFILKCFEPDPDKRACANDLLVDEFLKVSSKKKKTQPKL
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Vector:pLIC-SGC1

Growth

Medium:
Antibiotics:
Procedure:1 ml from a 10 ml overnight culture containing 100 µg/ml kanamycin was used to inoculate 1 liter of TB media containing 100 µg/ml kanamycin. Cultures were grown at 37°C until the OD600 reached ~2.0. After that the temperature was adjusted to 25°C. Expression was induced for 4 hours using 1mM IPTG. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 300 mM NaCl; 20 mM imidazole.

Purification

Procedure
Column 1: Ni-affinity chromatography.
The column was equilibrated with binding buffer. The lysate was applied to the column which was subsequently washed with wash buffer 1. MAP3K5 was eluted with elution buffer. The eluted protein was collected and analyzed by SDS-PAGE. DTT was added to the protein sample

to a final concentration of 5mM. The N-terminal his 6 -tag was cleaved by incubating the protein overnight with TEV protease.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)
The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 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 0.8 ml/min. MAP3K5 eluted at a retention time corresponding to the monomeric protein. 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 sonication . The lysate was centrifuged at 19,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 13 mg/ml containing and 1 mM of staurosporine in SEC buffer. 100nl of the concentrated protein were mixed with 100nl of a well solution containing 80% MPD, 100 mM Hepes pH 7.8
NMR Spectroscopy:
Data Collection:X-ray source: Swiss Light Source beamline BL10, single wavelength.
Data Processing: