MAPK6: Human MAP Kinase 6 (ERK3)

PDB:2I6L

Revision

Revision Type:created
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Entry Clone Accession:NP_002739
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqSMNIHGFD LGSRYMDLKPLGCGGNGLVFSAVDNDCDK RVAIKKIVLTDPQSVKHALREIKIIRR
LD HDNIVKVFEILGPSGSQLTDDVGSLTELN SVYIVQEYMETDLANVLEQGPLLEEHARL FMYQLLRGLKYIHSANVLHRDLKP
ANLFI NTEDLVLKIGDFGLARIMDPHYSHKGHLS EGLVTKWYRSPRLLLSPNNYTKAIDMWAA GCIFAEMLTGKTLFAGAHELE
QMQLILES IPVVHEEDRQELLSVIPVYIRNDMTEPHK PLTQLLPGISREAVDFLEQILTFSPMDRL TAEEALSHPYMSIYSFPM
DEPI

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Grow starter cultures from freshly transformed colonies in 10 ml LB, 50 mg/ml kanamycin. This started culture was diluted 1:1000 in fresh media and was grown at 37°C to a OD600 of 0.4 and than transferred to 18°C. Expression was induced at an OD600 of 0.6 - 0.7 using 1 mM IPTG (final concentration). Cells were harvested after 4h by centrifugation (15min, 6500rpm on a JLA 8.100 rotor), transferred to 50-ml tubes, and frozen at -20°C.

Purification

Procedure
Column 1 : DE52/Ni-NTA
Gravity feed chromatography: A DE52 column (10 g suspended in 100ml of 2.5M NaCl) was equilibrated with 100ml of Loading Buffer. A 5ml NiNTA column was equilibrated with 20ml of Loading Buffer. The lysed sample was applied to the DE-52 column and washed through with 50 ml loading buffer. The flow through was applied to the 5 ml Ni-NTA column which was washed

with 2x10ml of wash buffer and eluted with elution buffer in 5 ml aliquots (Step elution using 50, 100, 150, 200 and 250 mM imidazole in the Elution Buffer)

Enzymatic treatment : Treated the IMAC elution(s) with TEV protease overnight.

Column 2 : SEC (AKTA-prime)
Fractions containing MAPK6A collected from IMAC and treated with TEV protease overnight (identified by SDS PAGE) were concentrated to about 1.5ml and directly applied to a S75 16/60 column equilibrated in 10 mM Hepes pH 7.5, 100 m NaCl. The flow rate was 1ml/min and the pure protein eluted at 55-70min.

Concentration : The combined samples from the SEC column (identified by SDS PAGE) were concentrated using centricons with 10 kDa cut off.

Extraction

Procedure
The cell pellets (5 gr wet wt) were re-suspended in 50 ml extraction buffer containing a Protease Inhibitor Coctail tablet (Roche), and lysed in a high pressure cell disrupter. The supernatant was centrifuged for 45 minutes at 53k g in a JA 25.5 rotor
Concentration:
Ligand
MassSpec:LC- ESI -MStof confirmed the correct mass of 36237 expected for this construct after TEV treatment.
Crystallization: Crystals were grown at 4°C in 600nl sitting drops mixing 300 nl of MAPK6A (20 mg/ml in 10mM Hepes pH 7.5, 100mM NaCl ,10mM DTT) with 300 nl of a solution containing 1.8M MgSO4 and 0.1M MES pH 6.7. Cryo protection was achieved by adding to the crystallization mix Ethylene Glycole (20% final w/v).
NMR Spectroscopy:
Data Collection:
Data Processing: