NME3

PDB:1ZS6

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:37693992
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal histag with integrated thrombin cleavage site: mgsshhhhhhssglvpr*gs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMICLVLTIFANLFPAACTGAHERTFLAVKPDGVQRRLVGEIVRRFERKGFKLVALKLVQASEELLREH
YAELRERPFYGRLVKYMASGPVVAMVWQGLDVVRTSRALIGATNPADAPPGTIRGDFCIEVGKNLIHGSDSVESARREIALWFRADE
LLCWEDSAGHWLYE

Vector:p24a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/ml of kanamycin at 37ºC and grown to an OD600 of 2.1. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in a LEX bubbling system.

Purification

Procedure
The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 3 ml Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4oC. The Ni-NTA column was washed with 150 ml of the wash buffer (10mM Tris pH7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 ml of the elution buffer (10mM Tris pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol. The protein concentration was estimated based on the extinction coefficient of the protein, 28000 at

280 nm. Five molar equivalents of ADP, 10 mM DTT and 5 mM MgCl2 were added to the purified protein before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 1 ml and the concentration of 50 mg/ml. About 30 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were suspended in 100 ml of the binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification.
Concentration:
Ligand
MassSpec:
Crystallization: Purified His-tagged NME3 was crystallized using the sitting drop vapor diffusion method. Crystals grew in one day when the protein (15 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 20% PEG 3350, 0.2 M tri-lithium citrate.
NMR Spectroscopy:
Data Collection:
Data Processing: