NRK1

PDB:2P0E

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC001366
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal Hexahistidine tag and thrombin cleavage sequence site: mgsshhhhhhssglvpr*gs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

mgsshhhhhhssglvpr*gsKTFIIGISGVTNSGKTTLAKNLQKHLPNCSVISQDDFFKPESEIETDKNGFLQYDVLEALNMEKMMS
AISCWMESARHSVVSTDQESAEEIPILIIEGFLLFNYKPLDTIWNRSYFLTIPYEECKRRRSTRVYQPPDSPGYFDGHVWPMYLKYR
QEMQDITWEVVYLDGTKSEEDLFLQVYEDLIQEL

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: The target was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium containing 50 mg/L kanamycin and 50 mg/L chloramphenicol into a 1.8 L of Terrific Broth medium containing 50 mg/L kanamycin and 50 mg/L chloramphenicol. The culture was grown at 37ºC with the LEX bubbling system. When OD600 was ~3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15ºC, and the cells were allowed to grow overnight. Cultures were harvested by centrifugation and the cell pellets were flash frozen and stored at -80˚C.

Purification

Procedure
Column 1: DE52 column

Column 2: 5 mL Ni-NTA column (Qiagen)

Column 3: Superdex 200 column (26x60, Amersham Biosciences)

The lysate was centrifuged at 19000 xg for 30 min and the supernatant was passed through DE52 (Whatman) column (15mL bed volume) equilibrated with the binding buffer and the flow through was loaded onto 5 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4 ºC. The Ni-NTA column was washed with 150 mL of the washing buffer and the protein was eluted with 15 mL of the elution buffer. The protein was further purified and desalted using a gel filtration column, Superdex 200 (26/60), which was pre-equilibrated with Gel filtration buffer.

The protein was concentrated using an Amicon Ultra centrifugal filter with 5 kDa cut off to a final concentration of 30 mg/mL. Protein concentrations were measured using Bradford assay and the purity was >95% based on SDS-PAGE analysis. The His tag was cleaved using 1 unit of thrombin (Sigma T9681) per milligram of protein.

Extraction

Procedure
The thawed cell pellets from 4L were resuspended in 100 mL of the Lysis buffer with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride), and 0.5% CHAPS. The cells were lysed by microfluidizer at 20,000 psi.
Concentration:40 mg/mL
Ligand
MassSpec:
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method at 18ºC. The protein (40mg/mL) containing 10mM Tiazofurin, 1mM ADP and 20mM MgCl2 was equilibrated against a reservoir solution (1:1 volume ratio) containing 25% PEG3350, 0.2M sodium phosphate monobasic and 0.1M Bicine pH 9.0. The crystals reached a size of 100 microns within a week and were cryoprotected by washing them with a 1:1 Paratone-N : mineral oil mixture. The crystals then were frozen by directly immersing them into liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: