NEK2: Human never in mitosis gene A-related kinase 2

PDB:2JAV

Revision

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Entry Clone Accession:NP002488
Entry Clone Source: Steve Smerdon NIMR, London UK
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Tag: Tag sequence: Non -cleavable C-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

Vector:pET22b

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 100 µg/ml ampicillin was used to inoculate 1 liter of LB media containing 100 µg/ml ampicillin. Cultures were grown at 37°C until the OD600 reached ~0.7. After that the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1mM IPTG. The cells were collected by centrifugation and the pellets were frozen.

Purification

Procedure
Column 1: Ni-affinity chromatography.
Procedure: The lysate was bound onto 5 ml of Ni-NTA Sepharose pre-equilibrated in lysis buffer. The resin was washed with 25 ml lysis buffer supplemented with 1 M NaCl, then with 25 ml of lysis buffer. Protein was eluted with lysis buffer supplemented with 150 mM imidazole. DTT was added to 5 mM, MnCl2 was added to an end concentration of 0.5 mM, Phosphates were removed adding 20 µl of Shrimp alkaline phosphatase and 100 µl of ?-phosphatase during an 12 h incubation at 4°C.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)
Procedure: The protein sample was concentrated by ultrafiltration (10K cutoff) and fractionated on a S75 superdex column equlibrated in 50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM NaPO4,

5 mM DTT, 5% glycerol. Eluted fractions were 95% pure as judged by SDS-PAGE, and confirmed by mass spectrometry as the unphosphorylated protein.

Protein concentration: Centricon with a 10kDa cut off in SEC-buffer

Extraction

Procedure
Cell pellets were resuspended in 25 ml of 50 mM HEPES pH 7.5, 5 mM Sodium phosphate, 300 mM NaCl, 20 mM Imidazole and 5% glycerol. The cells were lysed by sonication for a total of 3 min.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained at 4°C using vapor diffusion sitting drops at a protein concentration of 7.5 mg/ml containing 1 mM of pyrrole-indolinone compound (5-[(Z)- (5-Chloro- 2-oxo-1,2- dihydro-3H- indol-3-ylidene) methyl]-N- [2-(diethylamino) ethyl]-2,4-dimethyl-1H- pyrrole-3-carboxamide). The protein-ligand complex was crystallized by mixing protein and precipitant at ratios of 4:1, 3:1 and 2:1. The crystallization precipitant comprised 10% polyethylene glycol 6000, 200 mM MgCl2.
NMR Spectroscopy:
Data Collection: Crystals were vitrified in mother liquor supplemented with 25% ethylene glycol. Data were measured at SLS (Swiss Light Source).
Data Processing: