Entry clone accession: PBC021-B01

Construct (coding) sequence:
gMAAAVADEAVARDVQRLLVQFQDEGGQLLGSPFDVPVDITPDRLQLVCNALLAQE
DPLPLAFFVHDAEIVSSLGKTLESQAVETEKVLDIIYQPQAI

Vector: pET28-MHL

Tags and additions:

Host: BL21(DE3) V2R-pRARE2

Growth system: LEX

Growth method:

NLE1 native protein was expressed in E. coli at 37°C by inoculating 20 mL of overnight culture grown in Luria-Bertani medium with 40ug/ml kanamycin and 25ug/ml chloramphenicol into 1L Terrific Broth medium in the presence of 40 ug/mL kanamycin. When the OD600 of the culture reached ~1.5, the temperature was lowered to 15 degree and the culture was induced with 0.5 mM IPTG. The cells were let grow overnight before harvested by centrifugation (7,000 rpm Beckman JLA-8.1000 rotor 10 min) and flash frozen in liquid nitrogen and stored at -80°C.

Extraction buffer: 20 mM Tris pH 8.0, 500 mM NaCl, 5% Glycerol

Extraction procedure: The cell pellet was resuspended in extraction buffer and the cells disrupted by sonication for 10 mins at 5" on 10" off duty cycle at 90W output power.

Purification buffers:

Washing Buffer: 20 mM Tris pH 8.0, 250 mM NaCl, 5% Glycerol, 50 mM imidazole
Elution Buffer: 20 mM Tris pH8.0, 250 mM NaCl with 5% glycerol, 250 mM imidazole

Purification procedure:

The crude extract was cleared by centrifugation. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni^{2+}. The column was washed with 10 CV of washing and the protein was eluted with 25ml elution.

The protein was further purified to homogeneity by Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 ml/min.

TEV protease was added to combined fractions containing NLE1 protein overnight at 4°C.

The protein was loaded onto ion-exchange chromatography on Source 30 Q column, equilibrated with buffer 20mM Tris pH7.5, and eluted with linear gradient of NaCl up to 1 M concentration (20CV).

Protein yield: 34 mg/L

Stock Concentration: 35.9mg/ml

Mass spec characterization: measured mass is 10573.5Da

Crystallization:

Purified NLE1 was crystallized using sitting drop vapor diffusion method at 20°C by mixing equal volumes of protein and reservoir solution containing 2.5M NH_4SO_4, 0.1 M Tris pH8.5.