NEDD4L C2 domain

PDB:2NSQ
SGC Clone Accession:nedd4l.0001.0154; plate SDC087, well D8

Sequence:

gMATGLGEPVYGLSEDEGESRILRVKVVSGIDLAKKDIFGASDPYVKLSLYVADENRELALVQTKTIKKTLNPKWNEEFYFRVNPSN
HRLLFEVFDENRLTRDDFLGQVDVPLSHLPTEDPTMERPYTFKDFLLRPRSHKSRVKGFLRLKMAYMP

Growth

Medium:TB

Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 microG/ml of kanamycin at 37degC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15degC. The culture was centrifuged and the cell pellets were collected and stored at -80degC.

Purification

Procedure:
Column 1: TALON metal-affinity resin column (BD Biosciences)
Column 2: HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham)

The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4 degC. The column was washed with wash buffer A, wash buffer B and again wash buffer A, and the protein was eluted with elution buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol and concentrated by ultrafiltration.

Extraction

Procedure: The cell pellet was resuspended in lysis buffer inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.

Structure Determination

Crystallization: Crystals of NEDD4L-C2 were obtained by means of hanging drop vapor diffusion in 14% PEG 4000, 0.2 M NaOAc, pH 6.5 in room temperature (298K),with 1 mM DTT added as reducing agent and 20% ethylene glycol added as cryo protector.