NR4A1

PDB:2GBD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI|27894342
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal his tag with integrated thrombin protease site MGSSHHHHHHSSGLVPRGSHM
Host:E. coli BL21 (DE3) Codon Plus RIL (Stratagen)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgshmGRLPSKPKQPPDASPANLLTSLVRAHLDSGPSTAKLDYSKFQELVLPHFGKEDAGDVQQFYDLLSG
SLEVIRKWAEKIPGFAELSPADQDLLLESAFLELFILRLAYRSKPGEGKLIFCSGLVLHRLQCARGFGDWIDSILAFSRSLHSLLVD
VPAFACLSALVLITDRHGLQEPRRVEELQNRIASCLKEHVAAVAGEPQPASCLSRLLGKLPELRTLCTQGLQRIFYLKLEDLVPPPP
IIDKIFMDTLPF

Vector:p28a-thrombin

Growth

Medium:
Antibiotics:
Procedure:NR4A1 was expressed in E. coli BL21 (DE3) Codon Plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 12 oC.

Purification

Procedure
The crude extract was cleared by centrifugation. 5 mM imidazole was added to the lysate. The sample was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole). 20 mM DTT was added to NR4A1 containing fractions. The protein was loaded on Superdex 200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 200 mM NaCl, at flow rate 4 ml/min. Purification yield is 24.3 mg of protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 °C. For the purification the cell paste was thawed and resuspended in lysis buffer (1× PBS, 0.5 M NaCl, 5% glycerol, 0.1 % CHAPS) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 18,000 psi.
Concentration:12 mg/ml
Ligand
MassSpec:expected mass = 30228 Da, measured mass = 30275.8 Da
Crystallization: Purified NR4A1 was crystallized using the hanging drop vapor diffusion method at 18 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 15 % PEG 6000, 0.1 M BisTris pH 6.4, 0.4 M Sodium thiocyanate, and 5 % Ethylenglycol.
NMR Spectroscopy:
Data Collection:
Data Processing: