RXR-gamma

PDB:2GL8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NM_006917
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal his tag with integrated thrombin protease site
MGSSHHHHHHSSGLVPR*GSHN
Host:E. coli BL21 (DE3) Codon Plus RIL (Stratagen)

Construct

Prelude:
Sequence:

ATSGHEDMPVERILEAELAVEPKTESYGDMNMENSTNDPVTNICHAADKQLFTLVEWAKRIPHFSDLTLEDQVILLRAGWNELLIAS
FSHRSVSVQDGILLATGLHVHRSSAHSAGVGSIFDRVLTELVSKMKDMQMDKSELGCLRAIVLFNPDAKGLSNPSEVETLREKVYAT
LEAYTKQKYPEQPGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLETPLQIT

Vector:pET28a

Growth

Medium:
Antibiotics:
Procedure:RXR-gamma 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 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 12 degC.

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 Ni^{2+}. 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). 10 mM DTT was added to RXR-gamma 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. Thrombin (Sigma) was added to combined fractions containing RXR-gamma and incubated overnight at 4°C. The protein was further purified to homogeneity by anion-exchange chromatography on Resource Q column (1 mL) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl pH 8.0 and eluted with linear gradient of NaCl up to 400 mM concentration (60CV). Purification yield is 12 mg of protein per 1L of culture.

N-terminal his tag was cleaved with thrombin protease (Sigma).

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:9.2 mg/ml
Ligand
MassSpec:expected mass = 26980.0 Da, measured mass = 26963.8 Da
Crystallization: Purified RXRG was crystallized using the hanging drop vapor diffusion method at 18 °C by mixing 2 µl of the protein solution with 2 µl of the reservoir solution containing 15 % PEG 3350 and 0.2 M CaCl2.
NMR Spectroscopy:
Data Collection:
Data Processing: