TLR10A

PDB:2J67

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC089406
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm.
Host:

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmKTTQEQLKRNVRFHAFISYSEHDSLWVKNELIPNLEKEDGSILICLYESYFDPGKSISENIVSF
IEKSYKSIFVLSPNFVQNEWCHYEFYFAHHNLFHENSDHIILILLEPIPFYCIPTRYHKLKALLEKKAYLEWPKDRRKCGLFWANLR
AAIN

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells (BL21(DE3)) from frozen glycerol stocks were grown in 20 mL of TB, supplemented with 8 g/L 87 % glycerol, 50 µg/mL kanamycin, at 37°C over night. The following morning, 20 ml of the over night cultures inoculated 1500 ml Terrific Broth with 8 g/L 87 % glycerol , 50 µg/mL kanamycin, and 100 µL BREOX. Cultivation was performed in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37°C until an OD600 nm of 2 was reached. The cultivations were down-tempered to 18 °C for 1h in a water bath. Expression of target protein was induced by addition of 0.5 mM IPTG and was allowed to continue over night at 18 °C.

Purification

Procedure
Columns:
HiTrap Chelating HP 1 ml (IMAC); HiLoadÂ 16/60 Superdex 200 Prep Grade (Gel filtration)

Purification was conducted automatically on an ÄKTA xpress system operated by UNICORN software at a flow of 0.8 ml/min. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating HP) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the HiTrap Chelating column and was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with 7.5 ml of IMAC Elution Buffer and loaded onto the Gel filtration column. The chromatogram from gel filtration showed one major protein peak that mainly consisted of TLR10A-h001 as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM. The protein was concentrated to 10.5 mg/ml and stored at -80ºC.

Extraction

Procedure
Cells were harvested by centrifugation (10 min, 5500 ×g) and pellets were resuspended in 50mM Sodium-Phosphate pH 7.5, 500mM NaCl, 10% glycerol, 10 mM Imidazole and 0,5 mM TCEP (IMAC Bind/Wash1 Buffer) supplemented with one tablet of Complete EDTA-free protease inhibitor tablet and freezed at -80 ºC.
The cells were quickly thawed in warm water and 4ul (1000 U) of Benzonase was added. Cells were disrupted by sonication and the lysate was centrifuged for 20 minutes at 49 000×g. The supernatant was decanted and filtered through 0.45µm filter prior to loading onto the ÄKTAxpress for furher purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystallization was performed using the vapour diffusion method with hanging drops containing 1 µl of protein solution (10.6 mg/mL) and 2 µl well solution (11% PEG 3350, 200 mM NaSCN), incubated at 20° C.
NMR Spectroscopy:
Data Collection:
Data Processing: