

CDC34

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Revision

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Entry Clone Accession:ubc54.BC018143.MGC.AT17-G3.pCMV-SPORT6

Entry Clone Source:

SGC Clone Accession:ubc54.007.184; plate SDC021B02

Tag:MGSSHHHHHHSSGLVPR*GS

Host:BL21 (DE3)

Construct

Prelude:

Sequence:

MGSSHHHHHHSSGLVPR*GSPSSQKALLLELKGLQEEPVEGFRVTLVDEGDLYNWEVAIFGPPNTYYEGGYFKARLKFPIDYPYSPP
AFRFLTKMWHPNİYETGDCISILHPPVDDPQSGELPSEWRNPTQNVRTILLSVISLLNEPNTFSPANVDASVMYRKWKESKGDRE
YTDIIRKQVLGTKVDAERDGVKVP

Vector:pET28a-LIC

Growth

Medium:

Antibiotics:

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

Purification

Procedure

The cleared lysate was loaded onto a TALON metal-affinity resin column (BD Biosciences) at 4°C (1.5 ml settled gel volume per liter original cell culture). The column was washed with 10 ml wash buffer A, 10 ml wash buffer B and then with 30 ml wash buffer A, and the protein was eluted with 6 ml elution buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with GF buffer and concentrated by ultrafiltration to a final protein concentration of 42 mg/ml using Amicon Ultra centrifugal filter with 10kD cutoff.

Extraction

Procedure

Cell pellets were resuspended in Lysis buffer (25 ml per liter culture), lysed using Microfluidizer at 18000 psi, and cleared by centrifugation.

Concentration:

Ligand

MassSpec:

Crystallization: Crystals were grown in hanging drops by mixing 2 μ l protein solution with 2 μ l well solution (28% PEG 4000, 0.1 M Tris-HCl, pH 8.5, 0.2 M MgCl₂, 1 mM DTT and 7.5 mM glycyl-glycyl-glycine) at 21°C. For cryoprotection, the crystals were soaked in well solution supplemented with 25% ethylene glycol.

NMR Spectroscopy:

Data Collection:

Data Processing: