RPRD1B

PDB:4FLD

Revision

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Entry Clone Accession:NP_067038.1
Entry Clone Source:MGC DNA 05-F5 (NP_067038.1)
SGC Clone Accession:RPRD1B (DCC020G07): 2-135
Tag: TagN-terminal tag: MHHHHHHSSGRENLYFQG
Host:BL21 (DE3) Codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQG SSFSESALEKKLSELSNSQQSVQTLSLWLIHHRKHAGPIVSVWHRELRKAKSNRKLTFLYLANDVIQN
SKRKGPEFTREFESVLVDAFSHVAREADEGCKKPLERLLNIWQERSVYGGEFIQQLKLSMEDSKSP

Vector:pET15-MHL

Growth

Medium:
Antibiotics:
Procedure:A 250 mL flask containing LB (Sigma L7658) supplemented with 50 µg/mL kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 °C. Using the Lex system, a 2L bottle (VWR 89000-242) containing 1800 mL of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ mL kanamycin and 600 µl antifoam 204 (Sigma A-8311) was inoculated with 50 mL overnight LB culture, and incubated at 37 °C. The temperature of the media was reduced to 15 °C one hour prior to induction and induced at OD600 = 6 with 100 µM isopropyl-thio-β-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 °C, and cell pellets collected by centrifugation and frozen at -80 °C.

Purification

Procedure
IMAC: Unclarified lysate was mixed with 2-3 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4oC. The mixture was then loaded onto an empty comLum (BioRad) and washed with 100 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280. pTEV was added to eluted protein at 1:20 for eluted protein and dialyze against gel filtration buffer overnight to remove His-tag.

Gel filtration chromatography: An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The dialyzed sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure
Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 25-40 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 30 mg/mL.
Ligand
MassSpec:
Crystallization: The apo RPRD1B CID domain was crystallized in a buffer containing 30% PEG550MME, 0.1M ammounium sulfate, 0.1M sodium cacodylate, pH 6.5; The RPRD1B was mixed with unmodified or phosphorylated Ser2 CTD in a ratio of 1:3 and put on ice for 30 minutes before crystallization. The RPRD1B- S2P complex was crystallized in a buffer containing 2.0 M ammonium sulfate, 5% isopropanol. The RPRD1B- unCTD complex was crystallized in a buffer containing 25% PEG-1500, 0.2M ammonium sulfate, 0.1M HEPES, pH 7.5. The crystals were soaked in a cryoprotectant consisting of 88% reservoir solution and 12% glycerol (v/v).
NMR Spectroscopy:

Data Collection:
Data Processing: