RASL12

PDB:3C5C

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC053734
Entry Clone Source:MGC: AT72-G11
SGC Clone Accession:HPC073-F03
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgPLEVNLAILGRRGAGKSALTVKFLTKRFISEYDPNLEDTYSSEETVDHQPVHLRVMDTADLDTPRNCER
YLNWAHAFLVVYSVDSRQSFDSSSSYLELLALHAKETQRSIPALLLGNKLDMAQYRQVTKAEGVALAGRFGCLFFEVSACLDFEHVQ
HVFHEAVREARRE

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling. The target proteins were expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the media was lowered to 15 °reeC and the culutre was induced with 0.5mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °reeC.

Purification

Procedure
The lysates were centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 °reeC for 1 hours. The supernants were then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 10 mL washing buffer once. The proteins bound to beads were eluted with elution buffer twice using 7.5 mL each time. The flow-through was collected and loaded onto Supderdex-75 gel filtration column. Eluted fractions were pooled and supplemented with 10 times protein

concentration of GDP and incubated at 4 °reeC overnight. The protein was then concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 95% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells were thawed and resuspended in 150 mL the extraction buffer with freshly added 0.5% CHAPS and 2mM BME(final concentration) and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15000 PSI.
Concentration:24.4 mg/mL
Ligand
GDP, Mg2+**MassSpec:**21398.30, expected 21397.04
Crystallization: Crystallization were setup using SGC, Red Wings (RW) screen kits, and also using in situ proteolysis method (Nature Methods (2007) v.4, p.1019) with 1:100 Elastase and 1:100 Endoproteinase Glu-C V8.
Micro crystals were seen within four days at multiple conditions:
SA05 SC04 SF04 RB01 RC01 RA05 RC09 RD09 RG07
Elastase SA05 SC04 SF04 RF11
Endoproteinase RH04 RD09 RF11
The condition with best looking crystals (Endoproteinase RD09) was optimized using 24-well plate, hanging drop. Diffracting crystal were obtained from condition: 0.1 M Tris pH 8.5, 28% PEG4000, 0.2 M MgCl2. No cryo used.
NMR Spectroscopy:
Data Collection:
Data Processing: