CARM1

PDB:4IKP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:40288288
Entry Clone Source: Open Biosystems
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host: Sf9

Construct

Prelude:
Sequence:

gRTEESSAVQYFQFYGYLSQQQNMMQDYVRTGTYQRAILQNHTDFKDKIVLDVGCGSGILSFFAAQAGARKIYAVEASTMAQHAEVL
VKSNNLTDRIVVIPGKVEEVSLPEQVDIIISEPMGYMLFNERMLESYLHAKKYLKPSGNMFPTIGDVHLAPFTDEQLYMEQFTKANF
WYQPSFHGVDLSALRGAAVDEYFRQPVVDTFDIRILMAKSVKYTVNFLEAKEGDLHRIEIPFKFHMLHSGLVHGLAFWFDVAFIGSI
MTVWLSTAPTEPLTHWYQVRCLFQSPLFAKAGDTLSGTCLLIANKRQSYDISIVAQVDQTGSKSSNLLDLKNPFFRYTGTT

Vector:pFBOH-MHL

Growth

Medium:
Antibiotics:
Procedure: Sf9 cells were infected with virus and incubated at 27 °C for 48-72 hours until cell viability drops to 70-80%.

Purification

Procedure
For purification the cell paste was thawed and 3000 U of benzonase (Novagen) were added. Cells were lyzed by brief sonication. The clarified lysate was loaded onto a 2 mL TALON column (Clonetech). The column was washed with 50 column volumes of 20 mM Tris-HCl buffer, pH 8.0, containing 500 mM NaCl, 5% cglycerol and 5 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 5% glycerol, 250 mM imidazole). The eluted protein was loaded on a Superdex200 column (GE Healthcare), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl. Pooled fractions containing CARM1 were subjected to TEV treatment to remove His-tag. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (GE Healthcare), equilibrated

with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV).

Extraction

Procedure
Cells were harvested by centrifugation at 4,000 rpm at 4 °C for 15 minutes. After removing the medium, cells were washed with cold 1X PBS. PBS was removed after centrifugation and the pellet was resuspended in suspension buffer (20 mM Tris, pH 8.0, 500 mM NaCl, 5% glycerol, 2 mM ß-mercaptoethanol, 0.6 % NP-40, 1 X protease inhibitor cocktail (Roche), 5 mM imidazole The cell pellets were frozen in liquid nitrogen and stored at -80°C.
Concentration:10 mg/ml - Enzymatic treatmetn: TEV
Ligand
MassSpec:expected mass is 38763. 3 Da, measured mass is 38764.473 Da.
Crystallization: Purified CARM1 (8 mg/mL) was complexed with inhibitor at 1:5 molar ratio of protein:inhibitor and crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG3350, 0.2 M di-ammonium tartrate.
NMR Spectroscopy:
Data Collection:
Data Processing: