C9orf114

PDB:4RG1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:38679912
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: 6XHis-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gAEKEDRGRPYTLSVALPGSILDNAQSPELRTYLAGQIARACAIFCVDEIVVFDEEGQDAKTVEGEFTGVGKKGQACVQLARILQYL
ECPQYLRKAFFPKHQDLQFAGLLNPLDSPHHMRQDEESEFREGIVVDRPTRPGHGSFVNCGMKKEVKIDKNLEPGLRVTVRLNQQQH
PDCKTYHGKVVSSQDPRTKAGLYWGYTVRLASCLSAVFAEAPFQDGYDLTIGTSERGSDVASAQLPNFRHALVVFGGLQGLEAGADA
DPNLEVAEPSVLFDLYVNTCPGQGSRTIRTEEAILISLAALQPGLTQAGARHT

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:C9orf114 protein was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cells were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation. The lysate was loaded onto 5 ml HiTrap column (GE Healthcare), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl pH 8.0, containing 250 mM NaCl, 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (GE Healthcare), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl. The fractions containing C9orf114 were pooled and TEV protease was added 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 (20 CV).

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 °C. For purification, the cell paste was thawed and resuspended in lysis buffer buffer (1X PBS, 250 mM NaCl, 2 mM ß-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:13.1 mg/mL - Enzymatic treatment: TEV
Ligand
MassSpec: The expected mass for C9orf114 is 34299.7 Da, measured mass is 34376.48 Da.
Crystallization: Purified C9orf114 protein (10.2 mg/mL) was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH 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 28% PEG 3350, 0.2M di-NH4tart.
NMR Spectroscopy:
Data Collection:
Data Processing: