GCN5L2

PDB:1Z4R

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi 10835101
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21(DE3) Codon PlusRIL (Stratagene).

Construct

Prelude:
Sequence:

gsGIIEFHVIGNSLTPKANRRVLLWLVGLQNVFSHQLPRMPKEYIARLVFDPKHKTLALIKDGRVIGGICFRMFPTQGFTEIVFCAV
TSNEQVKGYGTHLMNHLKEYHIKHNILYFLTYADEYAIGYFKKQGFSKDIKVPKSRYLGYIKDYEGATLMECELNPRIPYT

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Column 1: The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10CV of wash buffer (20mM Tris-HCl, pH 8.0, 500 mM NaCl, 50 mM imidazole), and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5% glycerol, 250 mM imidazole).

The purified protein was dialyzed against buffer 20 mM HEPES-NaOH, pH 7.5, 150 mM NaCl and treated with thrombin (Sigma) overnight at 4oC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM HEPES-NaOH, pH 7.5, and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 20 mg of the protein per 1L of culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were flash frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (phosphate buffer saline (PBS), pH 7.5, 0.5 M NaCl, 5% glycerol) with protease inhibitor (0.1µM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.).
Concentration:
Ligand
MassSpec:
Crystallization: Purified GCN5L2 was complexed with acetylcoenzyme A (AcCoA) at 1:5 molar ratio of protein:AcCoA and crystallized using the sitting drop vapor diffusion method. Crystals grew in condition 42 of Wizard I Crystallization screen (Emerald BioSystems), containing 15% (v/v) ethanol, 100 mM Tris pH 7.0
NMR Spectroscopy:
Data Collection:
Data Processing: