MAGI1

PDB:2R4H

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:n/a
Entry Clone Source: Synthetic
SGC Clone Accession:
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMDFYTVELERGAKGFGFSLRGGREYNMDLYVLRLAEDGPAERSGKMRIGDEILEINGETTKNMKH
SRAIELIKNGGRRVRLFLKRGETSV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: An overnight culture (10 ml) was used to innoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin). The cells were cultured at 37°C with vigorous shaking (160 rpm) until the culture reached an OD600 of 1.5. At that point temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and cultured further for 18 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet of 1L was resuspended in 20 ml of lysis buffer and stored at -20°C until further use. Lysis buffer : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole, Complete TM EDTA-free protease inhibitor (Roche, 1tablet / 50ml).Gelfiltration bufffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5mM TCEP.

Purification

Procedure
Column 1: Ni-NTAA 0.5ml NiNTA column was equilibrated with 12 ml of Binding buffer. The lysed sample was applied to the column twice and washed through with 12 ml of Binding Buffer (Wash 1) and 25ml of Wash Buffer (Wash 2). The protein was eluted with 15mls of Elution

bufferColumn 2: Size Exclusion Chromatography (SEC) Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)AKTA Purifier Gel Filtration. The eluted fractions from the Ni-NTA column were loaded on the gel filtration column in Gelfiltration buffer at 1ml/min. The flow rate was 1ml/min and the pure protein eluted at 65-82min.

Extraction

Procedure
The resuspended pellet was thawed and lysed in a high pressure homogeniser and then centrifuged at 4°C for 45 minutes at 4.8g.
Concentration: The combined samples from the SEC column (identified by SDS PAGE) were concentrated using centricons with 5 kDa cut off (Amicon Ultra 5k, Millipore) to 1.5 mg/ml.
Ligand
MassSpec:LC-ESI-MS tof confirmed the correct mass of 12086 Da expected for this construct of MAGI1A.
Crystallization: The MAGI1A@9 was crystallized at 4°C using the sitting-drop vapor diffusion method. Diffraction quality crystals were obtained in a solution containing 20% PEG 3350, 0.1M citrate pH 5.5.
NMR Spectroscopy:
Data Collection: Crystals were cryoprotected using 25% glycerol and flash frozen in liquid nitrogen. Diffraction data were collected to 2.05 Å at the Swiss light source beam-line X10SA at a single wavelength of 1.006029 Å.
Data Processing: