MAGI1

PDB:2Q9V

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:gi|74272282
Entry Clone Source: Synthetic
SGC Clone Accession:MAGI1A-c036
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s.
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*sMEQDIFLWRKETGFGFRILGGNEPGEPIYIGHIVPLGAADTDGRLRSGDELISVDGTPVIGKSH
QLVVQLMQQAAKQGHVNLTVRQTRL

Vector:pNIC28-Bsa4.

Growth

Medium:LB
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 in 2 litres 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.

Purification

Procedure
Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)

AKTA Xpress Affinity/Gel Filtration. The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The

eluted peak of A280nm was automatically collected.

AKTA Xpress Affinity/Gel Filtration. The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted protein was collected in 1.8 ml fractions in a 96 well bloc and analyzed by SDS-PAGE. Positive fractions were pooled for TEV cleavage.

TEV cleavage: The gel filtration fractions containing MAGI1A were pooled and 350 µl of TEV protease solution (1mg/ml)was added. The digestion was left overnight at 4°C and cleavage was examined by SDS page, before passing the mixture through Ni-NTA resin.

Extraction

Procedure
The resuspended pellet was thawed and homogenised by sonication and then centrifuged at 4°C in Beckman JA-25.50 rotor for 60 minutes at 21.000(rpm).
Concentration: The protein was concentrated to 8.1 mg/ml by using an Amicon Ultra 5k concentrator (Millipore).
Ligand
MassSpec: The experimentally determined mass of MAGI1A was 9.866Da, which corresponds to the theoretical mass.
Crystallization: Crystals were grown by vapor diffusion in sitting drops at 4°C. A sitting drop consisting of 50 nl protein (8 mg/ml) and 100 nl well solution was equilibrated against well solution containing 20% PEG 3350, 10% ethylene glycol, 200 mM sodium acetate, 100 mM BTP 8.5. The crystal was transferred to a cryo-protectant consisting of well solution supplemented with 15% glycerol, mounted in a loop, and flash-cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection:2.0 Å , X-ray source: Rotating anode, FR-E superbright.
Data Processing: