Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: SCRIB1A-c006

GenBank GI number: gi|32812254

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: N-terminal TEV-cleavable (at *) his-tag with the following sequence
mhhhhhhssgvdlgtenlyfq*s

Protein sequence:
MEELTLTILRQTGGLGISIAGGKGSTPYK
GDDEGIFISRVSEEGPAARAGVRVGDKLL
EVNGVALQGAEHHEAVEALRGAGTAVQMR
VWRERETSV

Host: BL21(DE3)-R3-pRARE2

Growth medium, induction protocol: An overnight culture (10 ml) was used to inoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin and 34 µg/ml of chloramphenicol). The cells were cultured at 37°C with vigorous shaking (160 rpm) until the culture reached an OD_{600} of 1.5. At that point the temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.2 mM, and cultured further for 14 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet from 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).

Extraction method: The resuspended pellet was thawed and lysed in a high pressure homogeniser and then centrifuged at 4°C for 45 minutes at 4.8g.

Column 1: Ni-NTA

Buffers: Binding buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 20 mM imidazole,0.5mM TECP; **Wash Buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 40 mM imidazole,0.5mM TECP; **Elution Buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole, 0.5mM TECP.

Procedure: A 1 ml Ni-NTA 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 15 ml of Elution buffer.

TEV cleavage: The Ni-NTA fractions containing SCRIB1A were pooled (58 mg in total) and 250 µl of TEV protease solution (6 mg/ml) was added. The digest was left overnight at 4°C and cleavage was examined by SDS-PAGE after passing the mixture through a gel filtration column.

Column 2: Size Exclusion Chromatography (SEC) Hiload 16/60 Superdex 200 prep grade 140 ml (GE/Amersham Biosciences)

Buffers: Gel filtration bufffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5mM TCEP.

Procedure: AKTA Purifier Gel Filtration. The eluted fractions from the Ni-NTA column were loaded on Hiload 16/60 Superdex 200 column in Gelfiltration buffer at 1 ml/min. The flow rate was 1 ml/min and the pure protein was eluted at 85-110 min.The protein was collected in a 96 well block and analyzed by SDS-PAGE.

Column 3: Ni-NTA

Procedure: The combined SCRIB1A (TEV cleaved) samples from the SEC column (identified by SDS PAGE) were loaded on a Ni-NTA resin for further purification.
Concentration: The eluted flow through fraction from the Ni-NTA resin were concentrated using centricons with 5 kDa cut off (Amicon Ultra 5k, Millipore) to 5.86 mg/ml.
Mass spectrometry characterization: LC-ESI-MS TOF confirmed the correct mass of 10203 Da, as expected for this construct of MAGI1A.
Crystallization: Crystals were grown by vapour diffusion at 20°C in 150nl sitting drops consisting of 1:1 mix of purified protein and mother liquor containing 20% (v/v) PEG1000, 0.15 M NH_4Cl pH 6.3 and 15% ethylene glycol.
Data Collection: Crystals were cryo-protected in mother liquor supplemented with 20% ethylene glycol and flash frozen in liquid nitrogen. **Resolution:** 1.3 Å; **X-ray source:** SLS beam X10SA.