

Materials and Method

Note: To our best knowledge, this should represent an accurate description of the materials and methods required to reproduce our work. If any of the content on this page is difficult to interpret or should you have trouble repeating our work, do not hesitate to [contact us](#) as soon as possible in order for us to provide additional information and advice.

Entry Clone Source: Synthetic
Entry Clone Accession: n/a
SGC Construct ID: MAST3A-c010
GenBank GI number: gi 51475014
Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]
Coding DNA sequence: ATGCACCATCATCATCATCATTCTTCTGGT GTAGATCTGGGTACCGAGAACCTGTACTTC CAATCCATGCGTCCGCCGATTGTGATTCAT AGCAGCGGCAAAAAATATGGCTTTAGCCTG CGCGCCATTGCGGTGTATATGGGTGATAGC GATGTTTATACCGTTCATCATGTGGTGTGG AGCGTGGAAGATGGCAGCCCGGCCAGGAA GCGGGCCTGCGTGCGGGCGATCTGATTACC CATATTAACGGCGAAAAGCGTGCTGGGCCTG GTTACATGGATGTGGTGGAAGTCTGCTG AAAAGCGGCAATAAAATTAGCCTGCGTACC ACCGCGCTGGAAAATACCGAAACCAGCGTT
Final protein sequence (tag sequence in lowercase): smRPPIVIHSSGKKYGFSLRAIRVYMGDSD VYTVHHVVWSVEDGSPAQEAGLRAGDLITH INGESVLGLVHMDVVELLLKSGNKISLRRT ALENTETSV The N-terminal 2 residues, sm, derive from the vector, following TEV protease cleavage of the hexahistidine tag.
Tags and additions: Cleavable N-terminal His6 tag.
Expression strain: BL-21(DE3)-R3-Rosetta (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).
Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.
Glycerol stock preparation: A number of colonies from the transformation were used to inoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.
Expression: A glycerol stock was used to inoculate 50 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 2x 1L of TB media (18 ml starter culture into each) containing 33 µg/ml kanamycin. After 3 hours the temperature was reduced to 20°C. After a further 30 minutes the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight.
Cell harvest: Cells were spun at 6238x g for 10 mins at 4°C. The cells were resuspended in 60 ml of Lysis Buffer with the addition of 0.2 mM PMSF. The resuspended cell pellet was placed in a -80°C freezer.
Lysis Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP
Cell Lysis: The resuspended cell pellet was lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.25 % and the cell debris and precipitated DNA were spun down (18000 rpm, JA18 rotor, 90 min).

Column 1: Ni-NTA (2 ml volume in a gravity-flow column).
Column 1 Buffers: Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP; Wash Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 25 mM Imidazole pH 7.4, 0.5 mM TCEP; Elution Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4, 0.5 mM TCEP
Column 1 Procedure: The clarified cell extract was passed through the column. The column was then washed with Binding Buffer (25 ml) and Wash Buffer (25 ml). The protein was eluted with 10 ml of Elution Buffer.
Column 2: S200 16/60 Gel Filtration.
Column 2 Buffers: GF Buffer: 25 mM Hepes pH 7.4, 150 mM NaCl, 0.5 mM TCEP
Column 2 Procedure: The wash buffer and elution buffer fractions from column 1 were combined and concentrated to 2.5 ml volume in a 3 kDa MW cutoff spin concentrator. The concentrated sample was injected onto an S200 16/60 column pre-equilibrated in GF Buffer at 1.0 ml/min. 1.75 ml fractions were collected.
TEV protease digestion: Gel filtration fractions containing MAST4A PDZ domain were combined. TEV protease was added and the sample left at 4 °C over the weekend.
Rebinding of impurities to Ni-NTA: The protein was passed through a gravity-flow column containing Ni-NTA resin (2 ml resin volume, pre-equilibrated into GF Buffer), eluting with 20 mM imidazole.
Mass spectrometry characterization: Measured: 10759.9; Expected: 10759.3
Protein concentration: The TEV protease cleaved protein was concentrated to 12.5 mg/ml (measured by 280 nm absorbance), distributed into aliquots and frozen at -80°C.
Crystallization: Crystals grew from a 1:1 ratio of protein to precipitant solution (0.2M Lithium Sulphate, 35% PEG4000, 0.1M Tris.HCl pH9), using the vapour diffusion method, at 20°C.
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 25% Ethylene glycol, and then flash frozen in liquid nitrogen. Data was collected at the Swiss Light Source, beamline X10.