Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4132071

SGC Construct ID: FDPSA-c003

GenBank GI number: gi|4503685

Vector: p11. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: N-terminal tag: `mgsshhhhhhssgrenlyfqghm`

Protein Sequence (after tag removal; mutation marked in red):

GHMNGDQNSDVYAQEKQDFVQHFSQIVRV
LTEDEMGHPEIGDAIARLKEVLEYNAIGG
KYNRGLTVVVAFRELVEPRKQDADSLQRA
WTVGWCVELLQAFFLVADDIMDSSLTRRG
QICWYQKPGVGLDAINDANLLEACIYRLL
KLYCREQPYYLNLIELFLQSSYQTEIGQT
LDLLTAPQGNVDLVRFTEKRYKSIVKYK**S**
AFYSFYLPIAAAMYMAGIDGEKEHANAKK
ILLEMGEFFQIQDDYLDLFGDPSVTGKIG
TDIQDNKCSWLVVQCLQRATPEQYQILKE
NYGQKEAEKVARVKALYEELDLPAVFLQY
EEDSYSHIMALIEQYAAPLPPAVFLGLAR
KIYKRRK

Host: BL21(DE3)

Growth medium, induction protocol: Overnight cultures in LB (10 ml with100 µg/ml ampicillin) were used to inoculate 1 litre of LB medium containing 100 µg/ml ampicillin. Cultures were grown at 37°C until they reached an OD_{600} of 0.6-0.8 and then induced with 1 mM IPTG. The temperature was adjusted to 18°C and expression was allowed to continue overnight. The cells were collected by centrifugation.

Extraction buffer, extraction method: The cell pellet was resuspended in 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol.and lysed using a high pressure cell disruptor. The lysate was centrifuged at 17,000 RPM for 30 minutes at 4°C and the supernatant was collected.

Column 1: 2ml Ni-NTA agarose

Buffers: Binding: 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP; **Wash:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, 5% glycerol, 0.5 mM TCEP; **Elution:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol, 0.5 mM TCEP.

Procedure: Approximately 75mls of bacterial lysate was loaded by gravity onto a 2ml Ni-NTA agarose columns pre-equilibrated with binding buffer. The columns were then washed twice with 30ml binding buffer, then twice with 12.5ml of wash buffer. Protein was then eluted with 12.5 ml of elution buffer and collected as 1.5ml fractions. Fractions containing purified protein were pooled and concentrated to a volume of less than 5mls using a Vivaspin concentrator with 10 kD MW cutoff

Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml

Buffers: 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP

Procedure: The concentrated protein was loaded onto the column at 1 ml/min using an AKTA purifier system . Eluted protein was collected in 1 ml fractions. All fractions containing pure protein were pooled and concentrated to 85.8 mg/ml using a Vivaspin concentrator with 10 kD MW cutoff.

Mass spectrometry characterization: Characterisation of the protein by mass spectrometry revealed MW of 40713 kDa after TEV cleavage, coinciding with the predicted mass of the T to S mutant.

Crystallisation: Risedronate was prepared as a 100 mM stock solution in 100mM Tris HCl pH 7.7. $MgCl_2$ was prepared as a 100mM aquaeus stock solution. Risedronate and $MgCl_2$ were added to the protein to a final concentration of 2 mM each and a final protein concentration of 15 mg/ml. Crystals were grown at 20°C in 300 nl sitting drops by mixing 100 nl of protein solution and 200 nl of precipitant consisting of 0.2M NH4Cl pH6.3, 20% PEG 6000, 10% Ethylene glycol. Crystals were mounted using 20% ethylene glycol as a cryoprotectant before flash freezing.

Data Collection: Resolution: 1.95Å; **X-ray source:** SLS.