**Entry clone source:** Purely Proteins

**Entry clone accession:** gi|90652860

**Final protein sequence:**
gplgspgipSRVLQAEELHEKALDPFLL
QAEFFEIPMNFVDPKEYDIPGLVRKNRY
KTILPNPHSRVCLTSPDPDDPLSSYINA
NYIRGYGGEEKVYIATQGPIVSTVADFW
RMVWQEHTPIIVMITNIEEMNEKCTEYW
PEEQVAYDGVEITVQKVIHTEDYRLRLI
SLKSGTEERGLKHYWFTSWPDQKTPDRA
PPLLHLVREVEEAAQQEGPHCAPIIVHC
SAGIGRTGCFIATSICCQQLRQEGVVDI
LKTTCQLRQDRGGMIQTCEQYQFVHHVM
SLYEKQLSHQS

**Vector:** pGEX-6P2. Details [PDF ]; Sequence [ FASTA ] or [ GenBank ]

**Tags and additions:** Tag sequence:
mspilgywkikglvqptrllleyleekye
hlyerdegdkwrnkkfelglefpnlpyyi
dgdvkltqsmaiiryiadkhnmlggcpke
raeismlegavldirygvsriayskdfet
lkvdflsklpemlkmfedrlchktylngd
hvthpdfmlydaldvvlymdpmcldafpk
lvcfkkrieaipqidkylksskyiawplq
gwqatfgggdhppksdlevlfq*gplgsp
gip

PreScission? (rhinovirus 3C)- protease cleavable (*) GST tag

**Host:** BL21 (DE3) phage resistant

**Growth medium, induction protocol:** Starter cultures from freshly transformed colonies in 10 ml LB, and ampicillin were grown overnight. This was diluted 1:1000 in fresh media (6L) and was grown at 37°C to an OD 600 of 0.3 and than transferred to 18°C. Expression was induced at an OD 600 of 0.8 using 1 mM IPTG. Cells were harvested after 3h by centrifugation, transferred to 50-ml tubes, and frozen in liquid nitrogen.

**Extraction buffer, extraction method: Extraction buffer:** 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 10 mM DTT. The cell pellets (20 g wet wt) were re-suspended in 50 ml extraction buffer, and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 60,000 rpm.

**Column 1:** Glutathione Sepharose 4B affinity, 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

**Buffers: Binding buffer:** 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 10 mM DTT.

**Procedure:** Supernatant was applied at gravity flow, followed by a wash with 30 ml binding buffer. The GST-fusion was cleaved while bound to the column by addition of PreScission protease. The column was gently rotated overnight a 4°C then protein eluated with 3 bed volumes of binding buffer.

**Column 2:** SEC

**Buffers:** 10 mM Tris-HCl, pH 8.0, 300 mM NaCl, 10 mM DTT.

**Procedure:** Akta-Prime

**Column 3:** HiTrap Q

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| **Protein concentration:** Centricons 10 kDa cut off |
| **Mass spec characterisation :** LC-ESI-MS TOF confirmed the correct mass expected for this construct (33252 Da). |
| **Crystallization:** Crystals were obtained using sitting drop method at 4°C. Drops were prepared using 150 nl of protein (10 mg/ml concentration) and 150 nl of the well solution (25% PEG 3350, 0.2M LiSO 4 , 100 mM Bis Tris Propane pH 5.5). |
| **Data Collection: Resolution:** 1.8 Å; X-ray source: Synchrontron BESSY BL1. |