

Entry clone accession BC053905

Entry clone source 35-G2

Tag N-terminal hexahistidine tag with integrated TEV protease cleavage site:
mhhhhhssgrenlyfqg.

Construct sequence Mhhhhhssgrenlyfqg
KRRRCGVCEPCQQTNCGECTYCKNRKNSHQICKRRKCEELKKKPS

Vector pET28-MHL

Expression host E. coli BL21(DE3)-V2R-pRARE2

Growth medium TB

Growth method A fresh transformation was used to inoculate 50 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium. The culture was grown in LEX at 37°C to OD600 of 3.1. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 16°C and the culture was incubated for a further 18 hours before harvesting the cells.

Extraction buffers Lysis buffer: 20mM Tris pH=8.0, 500mM NaCl, 5% Glycerol, 0.1% NP40, 1mM PMSF, 5 units/mL Benzonase nuclease, 0.2 mM β-mercaptoethanol.

Extraction procedure Cells were harvested by centrifugation and pellets were stored in -80°C. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.

Purification buffers NiNTA Elution buffer (EB): 20mM Tris-HCl pH 7.5, 500mM NaCl, 250 mM Imidazole

Gel Filtration buffer: 20mM Tris pH 7.5, 150mM NaCl, 1mM DTT.

Purification procedure Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4°C, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB. The Column 2: Gel Filtration (Superdex S75 16/60 Hi-Load, GE Healthcare). Then the fractions containing protein were identified on a SDS-PAGE gel.

Protein stock concentration 10mg/ml.

Crystallization 25.0% (w/v) PEG 3350, 0.2 M NH₄OAc and 0.1 M Hepes, pH 7.5