

Entry clone accession Tb427.10.8150:MAC054-H04:C234720

SGC clone accession Tb427.10.8150:MAC054-H04:C234720

Tag removed

Construct sequence

YLDTSFYKQCRSILHVVMDLDRDGIFARDPSKLPDYRMIISHPMWWDLIKARLTRYEYTS
PSAFINDMRLVVQNCYDYNREESPFSTLARRIEIAMEDLFVTELSL

Vector pET15-MHL

Expression host BL21(DE3)-CodonPlus-RIL-pACYC LamP

Growth medium TB with MgSO₄ and Trace Element

Growth method - do not change for all Express plasmid in E. coli BL21(DE3)-pRARE2 on LB agar (Luria broth) plate in the presence of ampicillin(100 µg/ml)+chloramphenicol (34 µg/mL). A single colony was inoculated into 25 mL of TB with ampicillin(100mg/ml)+chloramphenicol (34 mg/mL) in a 50 mL falcon tube and incubated with shaking at 220 rpm overnight at 37 °C. Then the culture was transfer into 1L of TB with ampicillin(100mg/ml)+chloramphenicol (34 mg/mL), 9ml 0.8M MgSO₄, 180ul trace element and 0.5 mL of antifoam (Sigma) in a 1 L bottle and cultured using the LEX system to an OD₆₀₀ of ~5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C

Extraction buffers - do not change Binding Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol

Extraction procedure - do not change The culture was harvested by centrifugation. Pellets from 1 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80oC were thawed overnight at 4 °C on the day before purification. Prior to lysis, each pellet from 1 L of culture was pretreated with protease inhibitors, 0.5% CHAPS and 500 units of benzonase. Each liter of cells were sonicated for effective time 5 minutes(about 120 watts, pulsed 10s on, 10s off) and the cell lysate was centrifuged using a Beckman JA-16.25 rotor at 16,000 rpms for an hour at 10 °C

Purification buffers - do not change Wash Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, and 5 % glycerol

Elution Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, and 5 % glycerol

Gel Filtration buffer: 20mM HEPES 7.5 and 150 mM NaCl.

Purification procedure - do not change The cleared lysate was loaded onto a 2 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 – 1.5 mL/min. After the lysate was loaded, the column was then washed with at least 200 mL of Wash Buffer. After washing, the protein was eluted with 15 mL of Elution Buffer and treated with 1mM TCEP.

The sample was then loaded onto a superdex 200 gel filtration column. The eluted protein was concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore) with a 10 kDa cutoff. The protein was concentrated to 14 mg/mL and flash frozen in N₂(l) and stored at -80C.

Protein concentration protein concentration:12 mg/ml

Crystallization The 12 mg/ml (0.95 mM) protein in the base buffer (100 mM NaCl, 0.5 mM TCEP, 20 mM HEPES pH7,5) was crystallized at 20°C in 30% PEGM5K, 0.2 M NH₄SO₄, 0.1 M MES pH 6.5, in vapor diffusion sitting drop method. Final concentration of 3% DMSO and 1 mM SGC-CBP30 (IUPAC name: 8-(3-chloro-4-methoxy-phenethyl)-4-(3,5-dimethyl-isoxazol-4-yl)-9-(2-(morpholin-4-yl)-propyl)-7,9-diaza-bicyclo[4.3.0]nona-1(6),2,4,7-tetraene) was added to the concentrated protein immediately prior to setting up the crystallization plate