Entry clone accession Tb427tmp.01.5000

SGC clone accession Tb427tmp.01.5000:M1-L125:B6

Tag His tag removed

Construct sequence
MSQNRQLLYPREEMVSLVRSLDRPQENGLFSQDVLLQYPELAESYTKVCPNRCDLATAA
DRAAKGAYGYDVQLTTLKEDIRLMVNNCILFNGAEGAYADAARTFEKFAMGKIDAYISQ
KVGGRRL

Vector pET15-MHL

Expression host BL21-CodonPlus(DE3)-RIL

Growth medium TB

Growth method Express plasmid in E. coli BL21-CodonPlus(DE3)-RIL on LB(Lauria broth) plate in the presence of carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL). A single colony was inoculated into 25 mL of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL) in a 50 mL falcon tube and incubated with shaking at 220 rpm overnight at 37 ºC. Then the culture was transfer into 1L of TB with carbenicillin(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 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC

Extraction buffers Binding Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol

Extraction procedure 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-25.25 rotor at 24,000 rpms for 30 minutes at 10 ºC

Purification buffers 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: 10 mM HEPES, pH 7.5, 500 mM NaCl and 5% glycerol

Purification procedure 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 his-tag was cleaved with Tev protease overnight at 4 ºC in the presence of 1 mM TCEP (Tris(2-Carboxyethyl) phosphine Hydrochloride). The cleaved sample was then applied to a 1 mL Ni-NTA column pre-equilibrated with binding buffer. The flow-through was collected and the column was rinsed with additional 5 mL of binding buffer

The his tag cleaved 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 15 mg/mL and flash frozen in N2(l) and stored at -80C. Proein was diluted to 7.5 mg/mL for crystallization.

Protein stock concentration protein concentration:15mg/ml in 20mM HEPES7.5 and 150 mM NaCl.

Crystallization The protein was crystallized at 20 ºC in 0.1M imidazole 6.5, 1M Sodium acetate trihydrate with bromosporine using the Sitting drop method.