Entry clone accession cgd4_1340:MAC04H-E04:C228715

SGC clone accession cgd4_1340:I300-Q450

Tag

Construct sequence
ISNEADYDWRNECLRILNLLRKEQNSFLFENPVLESNDLTEETKNRYKEVIPEACDYITIEK
RLNNKSNSKRQSTSNQKRKSTTANSKSNQTIENPHEFERLVKLIFSNCMIFNPNSGECKWI
YDSAKQSLNKFNNLWNKSNVFLLYSNSQ

Vector pET15-MHL

Expression host BL21(DE3)-V3R-pRARE2

Growth medium TB with MgSO4 and Trace Element

Growth method - do not change 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 ºC. 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 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC

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 ºC

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:10 mg/ml in 20mM HEPES7.5 and 150 mM NaCl.

Crystallization The protein was crystallized at 293 K in 2.5M ammonium sulfate, 0.1 M bis-tris propane pH 7.0. Bromosporine (ethyl (3-methyl-6-{4-methyl-3-[(methylsulfonyl)amino]phenyl} [1,2,4]triazolo[4,3- b]pyridazin-8-yl)carbamate) was added (final concentration of 1 mM)directly to the concentrated protein immediately prior to setting up the crystallization plate