Entry clone accession PF13_0238

SGC clone accession PF13_0238:K338-I726:B07

Tag His tag removed

Construct sequence
KKKIVDANIATETMIDINVGGAIFETSRHTLTQQKDSFIEKLLSGRHHVTRDKQGRIFLDR
DSELFRIILNFLRNPLTIPIPKDLSESEALLKEAEFYGIKFLPFPLVFCIGGFDGVEYLNSMEL
LDISQQCWRMCTPMSTKKAYFGSAVLNNFLYVFGGNNYDYKALFETEVYDRLRDVWY
VSSNLNIPRRNNCGVTSNGRIYCIGGYDGSSIIPNVEAYDHRMKAWVEVAPLNTPRSSAM
CVAFDNKIYVIGGTNGERLNSIEVYEEKMNKWEQFPYALLEARSSGAAFNYLNQIYVVG
GIDNEHNILDSVEQYQPFNKRWQFLNGVPEKKMNFGAATLSDSYIITGGENGEVLNSCHF
FSPDTNEWQLGPSLLVPRFGHSVLIANI

Vector pFBOH-MHL

Expression host SF9 cell, Spodoptera frugiperda

Growth medium HyQ SFX-insect

Growth method The protein was expressed in SF9 cells. The cells were infected with P3 viral stocks and incubated at 27˚C, 100 rpm for 48-72 hours, until the cell viability dropped to 70-80%.

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

Extraction procedure "Cells were harvested by centrifugation at 4,000 rpm, 4 °C for 15 minutes. The cell pellets were washed with cold binding buffer then each liter of pellet resuspend with 30 ml of cold 1X PBS, freshly add

1mM PMSF/Benzamidine then freeze in liquid nitrogen and stored at -80 degree. "

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: 00 mM HEPES, pH 7.5, 500 mM NaCl , 5% glycerol

Purification procedure The frozen cell pellet was thawed overnight and resuspended with 0.6% NP-40, protease inhibitor cocktail (Roche), 3000 U of benzonase. The cells disrupted by sonication for 5 mins at 5" on 7" off cycle at 100-120W output power, and the cell lysate was centrifuged using a Beckman JA-25.25 rotor at 24,000 rpms for 30 minutes at 10 ºC. 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 liquid nitrogen and stored at -80 degree. Proein was diluted to 5 mg/mL for crystallization.

Protein stock concentration protein concentration: 15mg/ml in 10mM HEPES7.5, 500 mM NaCl and 5% glycerol

Crystallization The protein was crystallized at 20 ºC in 1% w/v Tryptone, 0.001 M Sodium azide, 0.05 M HEPES sodium pH 7.0,12% w/v Polyethylene glycol 3,350 using the Sitting drop method