Entry Clone Source: Collaborator

Entry Clone Accession: n/a

SGC Construct ID: SDHLA-c019

GenBank GI number: gi|33694276

Vector: pET28a. Details [PDF]; Sequence [FASTA] or [GenBank]

Host: BL21 (DE3) pLysE

Tags and additions: N-terminal non-cleavable His-tag

Expressed sequence (vector-incorporated sequence in lowercase):
mgsshhhhhhssglvprgshmasmtggqq
mgrgsaMLPNTGRLAGCTVFITGASRGIG
KAIALKAAKDGANIVIAAKTAQPHPKLLG
TIYTAAEEIEAVGGKALPCIVDVRDEQQI
SAAVEKAIKKFGGIDILVNNASAISLTNT
LDTPTKRLDLMMNVNTRGTYLASKACIPY
LKKSKVAHILNISPPLNLNPVWFKQHCAY
TIAKYGMSMYVLGMAEEFKGEIAVNALWP
KTAIHTAAMDMLGGPGIESQCRKVDIIAD
AAYSIFQKPKSFTGNFVIDENILKEEGIE
NFDVYAIKPGHPLQPDFFLDEYPEAVSKK
VESTGAVPElacgrtrapppppplrsgc

Expression: BL21 glycerol stock harbouring the SDHLA-pET28a plasmid was inoculated into 50ml TB medium supplemented with 100µg/ml ampicilin and 34µg/ml chloramphenicol and grown overnight at 37°C, 200 rpm. 10ml overnight culture was inoculated into 4x 1L TB supplemented with 100µg/ml ampicilin and incubated at 37°C, 160rpm. At OD_{600} ~1.0, 0.2mM IPTG was added and the culture was incubated overnight at 18°C, 160rpm. The following morning cells were harvested by centrifuging for 10min at 6000rpm, and were resuspended in 70ml lysis buffer.

Extraction: Cells were lyzed by sonication, frozen and then re-thawed. The lysate was centrifuged at 12,000rpm for 20min.

Buffers:
Lysis Buffer: 20 mM Tris pH 8.0, 100 mM NaCl, 20% Glycerol, 50mM Imidazole, 1mM PMSF;
Wash Buffer: 20 mM Tris pH 8.0, 800 mM NaCl, 20% Glycerol, 50mM Imidazole, 1mM PMSF;
Elution buffer: 20 mM Tris pH 8.0, 500 mM NaCl, 20% Glycerol, 50mM Imidazole, 1mM PMSF;
Dialysis buffer: 20 mM Tris pH 8.0, 500 mM NaCl, 10% Glycerol, 1mM TCEP

Procedure: The clarified lysate was adjusted to [NaCl] ~800mM, incubated with 0.5 ml of Ni-NTA resin for 25 min and then loaded onto a gravity flow column. Ni-NTA was washed with 20ml wash buffer. Elution buffer was then added to the column and protein was collected in 5 ml fractions which were analyzed by SDS-PAGE. The pooled fractions containing the protein were then dialyzed into dialysis buffer overnight at 4°C.

Concentration: Using Amicon Ultra-15 concentrators with 10kDa cutoff the protein was concentrated to 5.3 mg/ml.
Mass specrometry characterization: The calculated mass of the tagged protein (36,999Da) was experimentally confirmed by mass spec analysis.
Crystallization with NADP and 17β-Estradiol: Prior to crystallization protein was supplemented with 1.5 mM NADP and 0.75 mM 17β-estradiol. Crystals were grown at 20°C in 150nl sitting drops mixing SDHLA (5.3 mg/ml) with reservoir solution containing 0.1 M BIS-TRIS pH 5.5, 25% PEG 3350 in a 2:1 ratio. Crystals were cryo-protected with 25% glycerol before flash-freezing in liquid nitrogen.
Data collection: Resolution: 2.25 Å; **Beamline:** Diamond Light Source beamline I24