Pf-KsgA (dimethyladenosine transferase, putative)

PDB:2H1R

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PF14_0156
Entry Clone Source: Plasmodium falciparum 3D7 genomic DNA
SGC Clone Accession:
Tag:N-terminal: His6-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

gQHLLKNPGILDKIIYAAKIKSSDIVLEIGCGTGNLTVKLLPLAKKVITIDIDSRMISEVKKRCLYEGYNNLEVYEGDAIKTVFPKF
DVCTANIPYKISSPLIFKLISHRPLFKCAVLMFQKEFAERMLANVGDSNYSRLTINVKLFCKVTKVCNVNRSSFNPPPKVDSVIVKL
IPKESSFLTNFDEWDNLLRICFSRKRKTLHAIFKRNAVLNMLEHNYKNWCTLNKQVPVNFPFKKYCLDVLEHLDMCEKRSINLDEND
FLKLLLEFNKKGIHFFNI

Vector:p15-tev-lic

Growth

Medium:
Antibiotics:
Procedure: Pf-KsgA was expressed in E. coli BL21-(DE3)-CodonPlus-RIL in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively). A single colony was inoculated into 100mL of LB with of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) in a 250 mL baffled flask and incubated with shaking at 250 rpm overnight at 37 ºC. The culture was transferred into 1.8 L of TB with ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of 4.5, cooled to 15 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer) and

the DE52 column was further washed with 20 mL of Binding Buffer. The lysate was subsequently loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1-1.5 mL/min. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 1-5 mM after approximately 15 more minutes. The eluted Pf-KsgA was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with Gel Filtration Buffer. The fractions corresponding to the eluted protein peak were collected. The His-tag was cleaved with TEV protease overnight at 4 ºC in the presence of 1mM DTT. The cleaved sample was applied to a 1ml Ni-NTA column pre-equilibrated with Binding buffer. The flow-through was collected; and the column was rinsed with an additional 5 mL of Binding Buffer. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Pf-KsgA was concentrated and flash frozen and stored at -80 ºC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Bufferwith the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at 80 oC were thawed overnight at 4 °C on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at 24000 rpms (~75000 x g) for 20 minutes at 10 oC.
Concentration: 17.1 mg/mL for Pf-KsgA (construct ID: PF14_0156:Q78-I355:B8, without histag).
Ligand
MassSpec:
Crystallization: Purified Pf-KsgA was crystallized using the hanging drop vapor diffusion method at 18 ºC. 1.5 µL of the protein solution was mixed with 1.5 µL of the reservoir solution containing 23.5% PEG3350, 0.2 M Tri-Lithium Citrate and 0.1 M Hepes pH7.1. Crystals appeared in 4-5 days.
NMR Spectroscopy:
Data Collection:
Data Processing: