FDPS (Farnesyl diphosphate synthase)

PDB:1YV5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:41281370
Entry Clone Source:MGC
SGC Clone Accession:
Tag:mgsshhhhhhssgrelyfqghm
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrelyfqghmNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGKYNRGLTVVV
AFRELVEPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYY
LNLIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTEKRYKSIVKYKTAFYSFYLPIAAAMYMAGIDGEKEHANAKKILLEMGEFFQ
IQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCLQRATPEQYQILKENYGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMA
LIEQYAAPLPPAVFLGLARKIYKRRK

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: Overnight cultures in TB (10 ml with100 µg/ml ampicillin) were used to inoculate 1 litre of TB medium containing 100 µg/ml ampicillin. Cultures were grown at 37oC until they reached an OD600 of 0.65 and then induced with 1 mM IPTG. The temperature was adjusted to 18oC and expression was allowed to continue overnight. The cells were collected by centrifugation, transferred to 50 ml tubes, resuspended in 25 ml binding buffer, and frozen at -80°C.

Purification

Procedure
Ni-affinity Ni-NTA (Qiagen), 4 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer. Binding buffer: 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol. The supernatant was applied by gravity flow onto the Ni-NTA column. The column was sequentially washed with

30 ml binding buffer and 12.5 ml wash buffer. The protein was eluted by applying 12.5 ml of elution buffer and the eluate was collected in 1.5 ml fractions. The fractions were analyzed by SDS-PAGE gel, pooled and concentrated. FDPS was exchanged into crystallization buffer (10 mM Hepes, 0.5 M NaCl, 5% glycerol, pH 7.5) and concentrated to 13 mg/mL using a Millipore centrifugal concentrator with a 10 kDa MW cutoff.

Extraction

Procedure
The frozen cells were thawed on ice and lysed using a high pressure cell disruptor. The lysate was centrifuged at 17,000 RPM for 30 minutes at 4°C and the supernatant was collected.
Concentration:
Ligand
MassSpec:
Crystallization: Risedronate and MgCl2 were prepared as 100 mM aqueous stock solutions and added to the protein to a final concentration of 2 mM each. Crystals were grown at 20oC in 150 nl sitting drops by mixing 100 nl of protein solution and 50 nl of precipitant consisting of 40% PEG 300 and 0.1 M phosphate-citrate buffer pH 4.2.
NMR Spectroscopy:
Data Collection:
Data Processing: