PYCS: human pyrroline-5-carboxylate synthetase 1 - aldehyde dehydrogenase domain

PDB:2H5G

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PYCSA-s001 (gi|21361368)
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal His-tag with TEV protease cleavage site (Tag sequence in lowercase)
Host:E.coli strain Rosetta

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMVKPAGP TVEQQGEMARSGGRMLATLEPEQRAEIIH HLADLLTDQRDEILLANKKDLEEAEGR
LA APLLKRLSLSTSKLNSLAIGLRQIAASSQ DSVGRVLRRTRIAKNLELEQVTVPIGVLL VIFESRPDCLPQVAALAIASGNGL
LLKGG KEAAHSNRILHLLTQEALSIHGVKEAVQL VNTREEVEDLCRLDKMIDLIIPRGSSQLV RDIQKAAKGIPVMGHSEGICH
MYVDSEAS VDKVTRLVRDSKCEYPAACNALETLLIHR DLLRTPLFDQIIDMLRVEQVKIHAGPKFA SYLTFSPSEVKSLRTEYG
DLELCIEVVDN VQDAIDHIHKYGSSHTDVIVTEDENTAEF FLQHVDSACVFWNASTRFSDGYRFGLGAE VGISTSRIHARGPVG
LEGLLTTKWLLRGK DHVVSDFSEHGSLKYLHENLPIPQRNTN

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:10 microL of a glycerol stock was inoculated into 5ml of LB medium (supplemented with Kanamycin, 50µg/ml) in a 15 ml culture tube and cultured at 37°C o/n in a shaking incubator (275 rpm). The starter culture was used to inoculate 100 ml of LB medium and was grown at 37°C (200 rpm). At an OD of 2.2 the culture was harvested and the cell pellet was washed twice with M9 minimal medium (Molecular Dimensions Ltd). The cells were resuspended and used to inoculate 1 liter of prewarmed minimal medium. Methionine synthesis was suppressed by addition of leucine, isoleucine and valine (dissolved as 50 mg/l for each aa) and lysine, threonine, and phenylalanine (100mg/l of each aa). Selenomethionine was added to a concentration of 25 mg/l, and the cultures were induced by supplementation with 1 mM IPTG. Cells were grown overnight at 18°C, collected by centrifugation and stored frozen until further use.

Purification

Procedure
Column : 1 ml HisTrap crude (GE/Amersham)

Concentration: 20 mg/ml using Vivaspin 10K concentrators

Extraction

Procedure
Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes . The clear supernantant was passed twice over a 2.5 ml Ni-NTA resin, washed and eluted with the specified buffers.
Concentration:
Ligand
MassSpec: Corresponds to theoretical mass, as determined by ESI - TOF MS .
Crystallization: Crystals were grown by vapor diffusion at 20°C in 1.6M $MgSO_4$ and 0.1M MES pH6.5. Ethylene Glycol (20% final concentration) was used as a cryoprotectant.
NMR Spectroscopy:
Data Collection: Resolution: 2.25Å X-ray source: Synchrotron SLS -X10
Data Processing: