

CYP11A1

PDB:3N9Z

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:AT51-H8 (BC032329)

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal: ferredoxin - MGC: BC010284; C-terminal: 6His-tag

Host:*E.coli* JM109 (Stratagene)

Construct

Prelude:

Sequence:

STRSPRPFNEIPSPGDNGWLNLYHFWRETGTHKVHLHHVQNFQKYGPITYREKLGNVESVYVIDPEDVALLFKSEGNPERFLIPPWV
AYHQYYQRPIGVLLKKSAAWKKDRVALNQEVMPEATKNFLPLLDVSRDFVSVLHRRIKKAGSGNYSGLDSDDLFRFAFESITNVI
FGERQGMLEEVNPEAQRFDIAIYQMFHTSVPMNLNPPDLFRLFRTKTWKDHVAAWDVIFSKADIYTQNFYWELRQKGSVHHDYRGI
LYRLLGDSKMSFEDIKANVTEMLAGGVDTTSMTLQWHL YEMARNLKVQDMLRAEVLAARHQAQGD MATMLQLVPLLKASIKETLR LH
PISVTLQRYLVNDLVLRDYMIPAKTLVQVAIYALGREPTFFFDPENFDPTRWLSKDKNITYFRNLGFGWGVQRCLGRRIAELEM TIF
LINMLENFRVEIQHLSDVGTTFNLILMPEKPISFTFWPFNQEATQQ

Vector:pCW-LIC-29

Growth

Medium:

Antibiotics:

Procedure:CYP11A1 was co-expressed with GroEL/ES in *E.coli* JM109 in TB medium. Cells were grown at 37°C to an OD₆₀₀ of 1.0 and induced by 0.5mM IPTG and 4mg/ml of arabinose and in the presence of 0.5mM δ -aminolevulinic acid and incubated 48 hours at 26°C.

Purification

Procedure

The lysate was centrifuged at 60 000g for 60min. The supernatant was loaded onto 5ml NiHiTrap column (Amersham Biosciences) equilibrated with Buffer A. The column was washed with buffer A and protein was eluted using a linear gradient of 5-100% Buffer B. The protein was further purified by ion-exchange chromatography on SourceQ column (Amersham Biosciences), equilibrated with 5 mM KPi, pH 7.4, 20% glycerol and 4 mM CHAPS and eluted with linear gradient of Buffer C.

Extraction

Procedure

Collected/resuspended cells in extraction buffer were disrupted in a high-pressure Microfluidizer (Microfluidics Corp.) at 18,000 psi. CHAPS was added to a final concentration 16 mM and lysate was incubated at 4°C for 60 min.

Concentration: 20 mg/ml

Ligand

MassSpec:

Crystallization: Purified CYP11A1 was crystallized in presence of cholesterol, 22R-hydroxycholesterol, 20S-hydroxycholesterol, and 20R,22R-dihydroxycholesterol using hanging drop vapor diffusion method drop at 18°C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 10% PEG 8K, 0.2 M calcium acetate, and 0.1 M HEPES pH 7.5.

NMR Spectroscopy:

Data Collection:

Data Processing: