CYP11A1

PDB:3N9Y

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:

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STRSPRPFNEIPSPGDNGWLNLYHFWRETGTHKVHLHHVQNFQKYGPIYREKLGNVESVYVIDPEDVALLFKSEGPNPERFLIPPWV
AYHQYYQRPIGVLLKKSAAWKKDRVALNQEVMAPEATKNFLPLLDAVSRDFVSVLHRRIKKAGSGNYSGDISDDLFRFAFESITNVI
FGERQGMLEEVVNPEAQRFIDAIYQMFHTSVPMLNLPPDLFRLFRTKTWKDHVAAWDVIFSKADIYTQNFYWELRQKGSVHHDYRGI
LYRLLGDSKMSFEDIKANVTEMLAGGVDTTSMTLQWHLYEMARNLKVQDMLRAEVLAARHQAQGDMATMLQLVPLLKASIKETLRLH
PISVTLQRYLVNDLVLRDYMIPAKTLVQVAIYALGREPTFFFDPENFDPTRWLSKDKNITYFRNLGFGWGVRQCLGRRIAELEMTIF
LINMLENFRVEIQHLSDVGTTFNLILMPEKPISFTFWPFNQEATQQ
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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 OD_{600} 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 4oC for 60min.
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 1µl of the protein solution with 1µl 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: