CYP7A1

PDB:3DAX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_000771
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal: MAKKTSS;
C-terminal: 5His-tag, no cleavage site
Host:*E.coli* JM109 (Stratagene).

Construct

Prelude:
Sequence:

```
makktssRRRQTGEPPLENGLIPYLGCALQFGANPLEFLRANQRKHGHVFTCKLMGKYVHFITNPLSYHKVLCHGKYFDWKKFHFAT
SAKAFGHRSIDPMDGNTTENINDTFIKTLQGHALNSLTESMMENLQRIMRPPVSSNSKTAAWVTEGMYSFCYRVMFEAGYLTIFGRD
LTRRDTQKAHILNNLDNFKQFDKVFPALVAGLPIHMFRTAHNAREKLAESLRHENLQKRESISELISLRMFLNDTLSTFDDLEKAKT
HLVVLWASQANTIPATFWSLFQMIRNPEAMKAATEEVKRTLENAGQKVSLEGNPICLSQAELNDLPVLDSIIKESLRLSSASLNIRT
AKEDFTLHLEDGSYNIRKDDIIALYPQLMHLDPEIYPDPLTFKYDRYLDENGKTKTTFYCNGLKLKYYYMPFGSGATICPGRLFAIH
EIKQFLILMLSYFELELIEGQAKCPPLDQSRAGLGILPPLNDIEFKYKFKHhhhhh
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Vector:pCW-LIC-29

Growth

Medium:
Antibiotics:
Procedure:CYP7A1 was co-expressed with GroEL/ES in *E.coli* JM109 in TB medium. Cells were grown at 37 degC 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
Column 1: 5ml NiHiTrap column (Amersham Biosciences)

Following the incubation 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 Source 30S

column (Amersham Biosciences), equilibrated with buffer 5mM KPi, pH 7.4, 20% glycerol, 7mM sodium chlorate and eluted with linear gradient of Buffer C.

Extraction

Procedure
Collected/resuspended cells with Lysis buffer were disrupted in a high-pressure Microfluidizer (Microfluidics Corp.) at 18.000 psi. The sodium chlorate was added to final concentration 23mM and lysate was incubated at 4°C for 60min.
Concentration:20 mg/ml.
Ligand
MassSpec: Expected MW is 56257, measured mass is 56255.
Crystallization: Purified CYP7A1 was crystallized in presence of cholesterol using hanging drop vapor diffusion method drop at 18 °C by mixing 1µl of the protein solution with 1µl of the reservoir solution containing 0.1 M Potassium chloride, 0.1 M tri-Sodium citrate pH 5.5, 20 % PEG 400.
NMR Spectroscopy:
Data Collection:
Data Processing: