CYP2R1

PDB:2OJD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:90199946
Entry Clone Source:LIFESEQ
SGC Clone Accession:
Tag:N-terminal: MAKKT; C-terminal: 4His-tag, no cleavage site
Host:

Construct

Prelude:
Sequence:

KQRRPMGFPPGPPGLPFIGNIYSLAASSELPHVYMRKQSQVYGEIFSLDLGGISTVVLNGYDVVKECLVHQSEIFADRPCLPLFMKM
TKMGGLLNSRYGRGWVDHRRLAVNSFRYFGYGQKSFESKILEETKFFNDAIETYKGRPFDFKQLITNAVSNITNLIIFGERFTYEDT
DFQHMIELFSENVELAASASVFLYNAFPWIGILPFGKHQQLFRNAAVVYDFLSRLIEKASVNRKPQLPQHFVDAYLDEMDQGKNDPS
STFSKENLIFSVGELIIAGTETTTNVLRWAILFMALYPNIQGQVQKEIDLIMGPNGKPSWDDKCKMPYTEAVLHEVLRFCNIVPLGI
FHATSEDAVVRGYSIPKGTTVITNLYSVHFDEKYWRDPEVFHPERFLDSSGYFAKKEALVPFSLGRRHCLGEHLARMEMFLFFTALL
QRFHLHFPHELVPDLKPRLGMTLQPQPYLICAERR

Vector:pCW-LIC-29

Growth

Medium:
Antibiotics:
Procedure:CYP2R1 was co-expressed with GroEL/ES in E.coli JM109 in TB medium with 100 µg/ml of ampicillin and 35 µg/ml of kanamycin. Cells were grown at 37oC to an OD600 of 1.0. Then 0.5 mM IPTG, 0.5mM ?-aminolevulinic acid and 4mg/ml of arabinose were added and incubation continued 48 hours at 29°C.

Purification

Procedure
Following the incubation the lysate was centrifuged at 60,000g for 60 min. The supernatant was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+ and equilibrated with 50 mM KPi, pH 7.4, containing 300 mM NaCl, 0.2% CHAPS and 20% glycerol (buffer A). The column was washed with 10 CV of buffer A and protein was eluted using a linear gradient of 5-100% Buffer B (buffer A+250mM imidazole). The protein was further purified by ion-exchange chromatography on Source 30S column (Amersham Biosciences), equilibrated with buffer 5 mM KPi, pH 7.4, 20% glycerol, 0.2% CHAPS and eluted with linear gradient of 0-50% Buffer C (50 mM KPi, pH 7.4, 20% glycerol and 1MNaCl).

Extraction

Procedure
Collected/resuspended cells (50 mM potassium phosphate, 300 mM NaCl, 0.2% CHAPS, 20% (v/v) glycerol, pH 7.4, 0.4mM PMSF) were disrupted in a high-pressure Microfluidizer (Microfluidics Corp.) at 18,000 psi. CHAPS was added to final concentration 1% and lysate was incubated at 4°C for 60min.
Concentration:20 mg/ml
Ligand
MassSpec: Expected MW is 54939, measured mass is 54940.5
Crystallization: Purified CYP2R1 was crystallized in presence of vitamin D3 using hanging drop vapor diffusion method drop at 18 °C by mixing 2 µl of the protein solution with 2 µl of the reservoir solution containing 1.1 M Ammonium sulfate, 0.1M ADA, pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: