DHRS1

PDB:2QQ5

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:gi|19923983
Entry Clone Source:synthetic
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag. Tag sequence: mhhhhhhssgrenlyfq(*)ghm
Host:E. coli BL21(DE3)-R3

Construct

Prelude:
Sequence:

ghmAPMNGQVCVVTGASRGIGRGIALQLCKAGATVYITGRHLDTLRVVAQEAQSLGGQCVPVVCDSSQESEVRSLFEQVDREQQGRL
DVLVNNAYAGVQTILNTRNKAFWETPASMWDDINNVGLRGHYFCSVYGARLMVPAGQGLIVVISSPGSLQYMFNVPYGVGKAACDKL
AADCAHELRRHGVSCVSLWPGIVQTELLKEHMAKEEVLQDPVLKQFKSAFSSAETTELSGKCVVALATDPNILSLSGKVLPSCDLAR
RYgs

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: Expression: 10 µl of BL21(DE3)-R3 glycerol stock were inoculated into 5ml of TB with 100µg/ml of ampicilin and 34µg/ml chloramphenicol and grown overnight at 37°C, 200 rpm. 10ml of overnight culture were added to 1L of TB with 100µg/ml ampicilin and incubated at 37°C, 160rpm. After the OD600 reached 1.0, the temperature was dropped to 18°C and 500µl of 1M IPTG was added to the final concentration of ~0.5mM. The culture was then incubated with shaking overnight at 18°C, 160rpm. The following morning the 8L culture was harvested and centrifuged for 10min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 160ml of a lysis buffer and frozen at -80°C.

Purification

Procedure
Purification: Column 1 : Ni-affinity, His-Trap, 1 ml (Amersham); Column 2: Superdex 200, HiPrep 16/60 (Amersham)Buffers: Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP; Washing buffer: 50mM HEPES pH 7.5,

500mM NaCl, 40mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP; Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 250mM Imidazole, 0.5mM TCEP; GF buffer: 10mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP.Procedure: The cell extract was loaded on the AKTA Express system The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. Positive fractions were pooled for TEV cleavage.TEV cleavage: TheHis-tag was cleaved with 1mg TEV per 40mg target protein at 4°C overnight. The protein was purified on IMAC Sepharose using buffers as above.

Extraction

Procedure
Extraction: Lysis buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml). The thawed cells were broken by 5 passes at 16.000 psi through a high pressure homogeniser followed by centrifugation for 45 min at 15.000rpm.
Concentration: Concentration and buffer exchange: Using Amicon Ultra-15 concentrators with 10kDa cutoff, the sample was buffer-exchanged into GF buffer and concentrated to 8mg/ml. Concentrations were determined from the absorbance at 280 nm using NanoDrop.
Ligand
MassSpec: Mass spec characterization: Calculated mass of the construct was 28485. The exact mass was confirmed by the mass spec.
Crystallization: Crystallization: Crystals were grown by vapor diffusion at 20°C in 300nl sitting drops. NADPH was added to a final concentration of 5mM prior to crystallisation. The drops were prepared by mixing 200nl of protein solution and 100nl of precipitant consisting of 2.4M Na malonate ph 7.0. Crystals were flash-cooled in liquid nitrogen with Na malonate acting as a cryo-protectant.
NMR Spectroscopy:
Data Collection: Data Collection: Resolution: 1.8Å; X-ray source: Rotating anode, FR-E superbright.
Data Processing: