

DHRS6

PDB:2AG5

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:DHRS6A-s001

Entry Clone Source:synthetic

SGC Clone Accession:

Tag:Histag with TEV cleavage site

Host:E.coli BL21 DE3

Construct

Prelude:

Sequence:

mgsshhhhhssgrenlyfqGHMGRLDGKVIILTAAQGIGQAAALAFAREGAKVIATDINESKLQELEKYPGIQTRVLDVTKKKQI
DQFANEVERLDVLFNVAGFVHHGTVLDCEEKDWD F SMNLNVRSMYLMIKAF L PKMLAQKSGNIINMSSVASSVKGVNRCVYSTTKA
AVIGLTKSVAADFIQQGIRCNCVCPGTVDTPSLQERIQARGNP EEARNDFLKRQKTGRFATAEEIAMLCVYLASDESAYVTGNPVII
DGGWSLGS

Vector:p11

Growth

Medium:

Antibiotics:

Procedure:The construct was expressed in E. coli in 1 L TB medium in the presence of 100 micro;g/mL of ampicillin at 37 o C. Cells were induced at 0.5 mM IPTG as soon as the OD reached 0.9, and temperature was shifted to 18 o C, and the culture was grown for 12 hrs. Cells were collected by centrifugation.

Purification

Procedure

Column 1 : Ni-NTA resin

Buffers (adjusted to pH 8.0): Lysis buffer: 5 mM Imidazole 500mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol. Wash buffer: 30 mM Imidazole, 500 mM NaCl 50mM, HEPES, pH 7.5, 5% glycerol. Elution Buffer: 250 mM Imidazole, 500 mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol.

Column 2: Superdex S200

Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 1mM TCEP

Procedure: Sample was loaded, washed with wash buffer and eluted in elution buffer. The protein was concentrated using an Amicon Ultra device.

Extraction

Procedure

Pellets were resuspended in 20 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, and the solution was centrifuged to obtain a clear supernatant (30 min, 20.000 x g). Supernatants were processed in a 2 step chromatographic procedure.

Concentration:

Ligand

MassSpec:

Crystallization: Crystals were obtained using the following conditions: Vapour diffusion method, sitting drop, 293K, 30% PEG 5K, 0.2 M ammonium sulfate, 0.1 M MES, pH 6.5.

NMR Spectroscopy:

Data Collection: Resolution: 1.86 Å , X-ray source: rotating anode (Rigaku FR-E SuperBright), single wavelength.

Data Processing: