

# 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:**

mgsshhhhhhssgrenlyfqGHMGRLDGVIIITAAQGIGQAAALAFAREGAKVIATDINESKLQELEKYPGIQTRVLDVTKKQI  
DQFANEVERLDVLFNVAGFVHHGTVLCEEKDWDFSMNLNVRSMYLMIKAFLPKMLAQKSGNIINMSSVASSVKGVVNRCVYSTTKA  
AVIGLTKSVAADFIQQGIRCNVCVCPGTVDTPSLQERIQARGNPEEARNDFLKRQKTGRFATAEEIAMLCVYLASDESAYVTGNPVII  
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 Imidazole500mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol. Wash buffer: 30 mM Imidazole, 500 mM NaCl50mM, 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 ammoniumsulfate, 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:**