

# AKR7A2

PDB:2BP1

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**AKR 7A2A-s001

**Entry Clone Source:**MGC

**SGC Clone Accession:**

**Tag:**N-terminal His-tag with TEV cleavage site: MHHHHHHSSGVDLG TENLYFQ\*SH

**Host:**E.coli BL21 Rosetta

## Construct

**Prelude:**

**Sequence:**

**Vector:**pLIC-SGC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**The AKR 7A construct was expressed in E. coli (BL21 Rosetta) in 1 L Terrific Broth in the presence of 100 mg/mL of ampicillin and 34 mg/mL chloramphenicol at 37 °C. Cells were induced at 1 mM IPTG as soon as the OD reached 0.6 and temperature was shifted to 25 °C, and culture was grown for 12 hrs. Cells were collected by centrifugation and pellets were stored frozen (-20 °C) until further use.

## Purification

**Procedure**

Column 1 : HiTrap His. Buffers (adjusted to pH 8.0): Lysis buffer: 10mM Imidazole, 300mM NaCl, 50mM NaH<sub>2</sub>PO<sub>4</sub>. Wash buffer: 20mM Imidazole, 300mM NaCl, 50mM NaH<sub>2</sub>PO<sub>4</sub>. Elution Buffer: 250mM Imidazole, 300mM NaCl. Procedure: Sample was loaded, washed with wash buffer and eluted in elution buffer. The collected peak was injected into size-exclusion chromatography system, and the main peak was selected for concentration using an Amicon Ultra device.

Column 2 : Superdex S200. Buffers : 10 mM Hepes, pH 7.4, 500 mM NaCl, 5% glycerol.

## Extraction

**Procedure**

Pellets were resuspended in 20 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, nucleic acids were removed by 0.15% PEI precipitation, and the solution was centrifuged to obtain a clear supernatant (15 min, 20.000 x g). Supernatants were processed in a 2 step chromatographic procedure using the Akta xpress (GE Healthcare) purification system.

**Concentration:****Ligand****MassSpec:**

**Crystallization:** Crystals in complex with NADPH were obtained using the following conditions: Vapour diffusion method, sitting drop, 293K, 0.2M Ammonium Citrate, 20% PEG 3350

**NMR Spectroscopy:****Data Collection:****Data Processing:**