Target ID: AKR1C4A

Entry clone source: MGC

Entry clone accession : BC020744

Vector: pNIC28-Bsa4

Tags and additions: N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s

Construct protein coding sequence (after TEV cleavage):

mhhhhhhssgvdlgtenlyfq*sMDPKYQRVELNDG
HFMPVLGFGTYAPPEVPRNRAVEVTKLAIEAGFRHI
DSAYLYNNEEQVGLAIRSKIADGSVKREDIFYTSKL
WCTFFQPQMVQPALESSLKKLQLDYVDLYLLHFPMA
LKPGETPLPKDENGKVIFDTVDLSATWEVMEKCKDA
GLAKSIGVSNFNYRQLEMILNKPGLKYKPVCNQVEC
HPYLNQSKLLDFCKSKDIVLVAHSALGTQRHKLWVD
PNSPVLLEDPVLCALAKKHKRTPALIALRYQLQRGV
VVLAKSYNEQRIRENIQVFEFQLTSEDMKVLDGLNR NYRYVVMDFLMDHPDYPFSDEY

Host : E.coli BL21(DE3)-R3

Growth medium, induction protocol: An overnight culture (10 ml each) was used to inoculate 6x 1L TB containing 50µg/ml of kanamycin. The cells were cultured at 37°C until the OD600 reached 1.00 and the temperature decreased to 18°C before IPTG was added to a final concentration of 0.5 mM. Growth was continued overnight and the cells were harvested by centrifugation.

Extraction buffer, extraction method : Extraction buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole. Cell pellets from 1 L cultures were resuspended in 25 ml of extraction buffer. The sample was homogenized by high-pressure using the EmulsiFlex-05 and clarified by centrifugation at 37,000 g

Column 1 : Ni-NTA

Buffers : **Binding buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole. **Wash buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 30 mM imidazole. **Elution Buffer:** : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole. **Procedure:** The column was packed with 4 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the pre-equilibrated column and column washed with 50 ml of binding buffer and then 50 ml of wash buffer. The AKR1C4A protein was eluted with 12 ml of elution buffer.

Column 2 : Superdex 200 HiLoad 16/60

Buffers : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 0.5 mM TCEP. **Procedure:** The Ni-NTA eluate was loaded onto the gel-filtration column and collected in 1 ml fractions. Fractions were analyzed by SDS-PAGE and pooled for further treatment.

Enzymatic treatment : His-tagged TEV protease (30 µg/mg protein) was added to the sample and incubated at 4°C overnight.

Column 3 : Ni-NTA

Buffers : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 0.5 mM TCEP. **Procedure:** After incubation with protease, the sample was passed through the Ni-NTA column and the flow through was collected.

Concentration : The protein was concentrated to 32 mg/ml using a 10,000 MW cutoff Amicon Ultra concentration device.

Mass Spec : The experimentally determined mass was 37,243, in close agreement with the predicted mass of 37,242.
Crystallization: Crystals were grown by vapor diffusion at 4°C in 150 nl sitting drops. NADPH to a final concentration of 5 mM was added to the protein just prior to crystallisation. The drops were prepared by mixing 75 nl of protein solution and 75 nl of precipitant consisting of 2M tri-potassium citrate. Crystals were transferred to a cryo-protectant consisting of 20% glycerol, 80 % well solution before flash-cooling in liquid nitrogen.
Data Collection: Resolution: 2.4Å, **X-ray source:** Rotating anode, Rigaku FR-E superbright