# CBR3: Human Carbonyl Reductase 3

**PDB:**2HRB

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**CBR3A-s001
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**
**Host:**E.coli strain BL21(DE3)-R3

## Construct

**Prelude:**
**Sequence:**

mhhhhhhssgvdlgtenlyfq*sMSSCSRVALVTGANRGIGLAIARELCRQFSGDVVLTARDVARGQAAVQQLQAEGLSPRFHQLDI
DDLQSIRALRDFLRKEYGGLNVLVNNAAVAFKSDDPMPFDIKAEMTLKTNFFATRNMCNELLPIMKPHGRVVNISSLQCLRAFENCS
EDLQERFHSETLTEGDLVDLMKKFVEDTKNEVHEREGWPNSPYGVSKLGVTVLSRILARRLDEKRKADRILVNACCPGPVKTDMDGK
DSIRTVEEGAETPVYLALLPPDATEPQGQLVHDKVVQNW

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**10 µl BL21(DE3)-R3 glycerol stock were inoculated into 5ml of TB with 50µg/ml of kanamycin and 34µg/ml chloramphenicol and grown overnight at 37°C, 200rpm. 10ml of overnight culture were added to 1L of TB with 50ug/ml kanamycin and incubated at 37°C, 160rpm. After the OD600 reached 1.0, the temperature was dropped to 18°C and 500 µl 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 4L culture was harvested and

centrifuged for 10min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 80ml of a lysis buffer and frozen at -80°C.

## Purification

**Procedure**
Column 1 : Ni-affinity, His-Trap, 1ml (Amersham)
Column 2 : SuperDex 200 16/60 HiLoad (GE/Amersham)
Buffers: Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP
Washing buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP
Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 30mM Imidazole, 0.5mM TCEP
GF buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP
Procedure (Columns 1 and 2): 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 : The His-tag was cleaved with 1 mg TEV per 40 mg target protein at 4°C overnight. The protein was purified on IMAC Sepharose using buffers as above.

Column 3 : Desalting column
Column 4 : Ion exchange QHP
Buffers: Buffer A: 50mM HEPES pH 7.5, 50mM NaCl, 0.5mM TCEP
Buffer B: 50mM HEPES pH 7.5, 2M NaCl, 0.5mM TCEP

Procedure (Columns 3 and 4): Desalted protein was purified in a 25% A/B gradient ran over 20 column volumes on QHP. A single resulting peak was characterised by the mass spec

Concentration: Concentration and buffer exchange: Using Amicon Ultra-15 concentrators with 10kDa cutoff, the sample was buffer-exchanged into GF buffer and concentrated to 10mg/ml. Concentrations were determined from the absorbance @ 280nm using NanoDrop.

## Extraction

**Procedure:** The thawed cells were broken by 5 passes at 16,000 psi through a high pressure homogeniser followed by centrifugation for 45min at 15,000rpm.
**Concentration:**
**Ligand:**
**MassSpec:** Calculated mass of the construct was 30937. The exact mass was confirmed by the mass spec
**Crystallization:**
**NMR Spectroscopy :**

**Data Collection:** Resolution: 1.9Å; X-ray source: Rotating anode, Rigaku FR-E superbright .
**Data Processing:**