# GLRX2

**PDB:**2FLS

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**BC009669
**Entry Clone Source:**Invitrogen LLAM 3879347
**SGC Clone Accession:**
**Tag:**Tag sequence: mhhhhhhssgvdlgtenlyfqs*(m), TEV-cleavable (*), N-terminal his6 tag.
**Host:**Rosetta-R3

## Construct

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

mhhhhhhssgvdlgtenlyfqsmPVNQIQETISDNCVVIFSKTSCSYCTMAKKLFHDMNVNYKVVELDLLEYGNQFQDALYKMTGER
TVPRIFVNGTFIGGATDTHRLHKEGKLLPLVHQCYLKKSKRKEFQ

**Vector:**pNIC28-BSA4

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Medium: TB + 50 µg/ml Kanamycin + 34 ug/ml chloramp . 2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 10 ml overnight culture and grown grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 4.5 at 18°C overnight . The cells were collected by centrifugation and frozen at -80°C.

## Purification

**Procedure**
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences )
Buffers: Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole; Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole; Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 250 mM imidazole.
Procedure: The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280nm was automatically collected.

Column 2: Hiload 16/60 Superdex 75 prep grade 120 ml (GE/Amersham Biosciences)

## Extraction

**Procedure**
Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, Complete® protease inhibitors (1 tablet/50 ml). Frozen cell pellets were thawed on ice overnight and resuspended in a total volume of 100 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40 000xg. The supernatant was further clarified by filtration (0.20 m m).
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences )
Buffers: Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole; Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole; Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 250 mM imidazole.
Procedure: The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280nm was automatically collected.

Column 2: Hiload 16/60 Superdex 75 prep grade 120 ml (GE/Amersham Biosciences)
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**