# Np95-like ring finger (NIRF) protein isoform b

**PDB:**1Z6U

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**
**Entry Clone Source:**MGC
**SGC Clone Accession:**ubh14.672.802; plate SDC013:C4
**Tag:**N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPRGS.
**Host:**E.coli BL21 (DE3)

## Construct

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

gsPSASKVYKASDSAEAIEAFQLTPQQQHLIREDCQNQKLWDEVLSHLVEGPNFLKKLEQSFMCVCCQELVYQPVTTECFHNVCKDC
LQRSFKAQVFSCPACRHDLGQNYIMIPNEILQTLLDLFFPGYSKGR

**Vector:**p28a-LIC

## Growth

**Medium:**TB
**Antibiotics:**
**Procedure:**Using the SGC LEX bubbling system, Np95-like ring finger protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37ºC to an $OD_{600}$ of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

## Purification

**Procedure**
The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4ºC. The column was washed with wash buffer A (10mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM ß-mercaptoethanol), wash buffer B (same as wash buffer A but containing 0.05% Tween 20) and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM ß-mercaptoethanol. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol and concentrated by ultrafiltration.

## Extraction

**Procedure**
The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM ß-mercaptoethanol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
**Concentration:**68 mg/mL
**Ligand**
**MassSpec:**
**Crystallization:**Purified Np95-like ring finger protein 3 was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (34 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 0.9M sodium citrate, 0.1M Tris-HCl, pH 8.5.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**