# ITSN2

**PDB:**3GF9

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**BC146779
**Entry Clone Source:**OpenBiosystems EHS1001-99608273
**SGC Clone Accession:**HPC098-A03
ITSN2:C1103-E1379
**Tag:**N-terminal tag: mhhhhhhssgrenlyfq*g
**Host:**BL21-CodonPlus(DE3)-V2R pRARE2

## Construct

**Prelude:**Tag not removed
**Sequence:**

```
mhhhhhhssgrenlyfqgCQVIAMYDYAANNEDELSFSKGQLINVMNKDDPDWWQGEINGVTGLFPSNYVKMTTDSDPSQQWCADLQ
TLDTMQPIERKRQGYIHELIQTEERYMADLQLVVEVFQKRMAESGFLTEGEMALIFVNWKELIMSNTKLLKALRVRKKTGGEKMPVQ
MIGDILAAELSHMQAYIRFCSCQLNGAALLQQKTDEDTDFKEFLKKLASDPRCKGMPLSSFLLKPMQRITRYPLLIRSILENTPESH
ADHSSLKLALERAEELCSQVNEGVREKENSDRLE
```

**Vector:**pET28-mhl (GI:134105571)

## Growth

**Medium:**Terrific Broth
**Antibiotics:**Kanamycin 50 µg/mL + Chloramphenicol 25 µg/mL
**Procedure:**LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 degC.

## Purification

**Procedure**
The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatant was mixed with 6 mL 50% Ni-NTA beads, and incubated at 4 degC on roller drum for 1 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 50 mL binding buffer followed by 50mL washing buffer. The protein bound

to beads were then eluted using 15 mL elution buffer. The flow-through was collected and loaded onto Supderdex-75 26/60 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000 ). The purity of the proteins was higher than 95% judged by SDS-PAGE.

## Extraction

**Procedure**
Frozen cells from 6L culture were thawed and resuspended in 400 mL Binding buffer with freshly added final concentration of 1mM PMSF/Benzamidine, 0.5% CHAPS and 5U/mL Benzonase (Sigma Catalog # E1014, 250U/µL), and supplemented with 1mL protease inhibitor cocktail (SIGMA Catalog # P8849), and lysed using sonication at 10 seconds 50% duty cycle for 6 minutes at 120W.
**Concentration:**31.1 mg/mL
**Ligand**
**MassSpec:**Native: 33976.13, expected 33975.77
**Crystallization:**Stock protein solution was mixed with 1:100 (m/m) papain and then crystallization was setup in sitting drops with Red Wings and SGC-I screens kits. Rod-shaped crystals were seen at multiple PEG conditions, includes RW-A2 and SGC-G9. Crystal used for data collection was grown in 25% PEG 3350, 0.1M (NH4)2SO4, 0.1M Bis-Tris pH 5.5. Sitting drop setup. 0.3 µL protein (include 1:100 papain) + 0.3 uL well solution equilibrated with 100 µL buffer.

SDS-PAGE gel shows the protein in the crystal has a size of 23 kDa, which is about 10 kDa smaller than expected. The original construct include an SH3 and an GEF domain, only the GEF domain is crystallized.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**