# PTPTN5

**PDB:**2BIJ

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:** gi 22095375
**Entry Clone Source:**Origine
**SGC Clone Accession:**
**Tag:**N-terminal His-tag with integrated TEV protease site:
MHHHHHHSSGVDLGTENLYFQ*SH
**Host:**BL21 (DE3)

## Construct

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

mhhhhhhssgvdlgtenlyfqsmSRVLQAEELHEKALDPFLLQAEFFEIPMNFVDPKEYD IPGLVRKNRYKTILPNPHSRVCLTSP
DPDDPLSSYINANYIRGYGGEEKVYIATQGPIVS TVADFWRMVWQEHTPIIVMITNIEEMNEKCTEYWPEEQVAYDGVEITVQKVI
HTEDYRLR LISLKSGTEERGLKHYWFTSWPDQKTPDRAPPLLHLVREVEEAAQQEGPHCAPIIVHCSA GIGRTGCFIATSICCQQ
LRQEGVVDILKTTCQLRQDRGGMIQTCEQYQFVHHVMSLYEKQ LSHQS

**Vector:**pLIC-SGC

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Grow starter cultures from freshly transformed colonies in 10 ml LB , 0.1 mg/mL amp. This started culture was diluted 1:1000 in fresh media and was grown at 37oC to a OD600 of 0.3 and than transferred to 18 oC. Expression was induced at an OD600 of 0.8 using 1 mM IPTG. Cells were harvested after 3h by centrifugation, transferred to 50-ml tubes, and frozen in liquid nitrogen.

## Purification

**Procedure**
Ni affinity, HisTrap (1 ml), in AKTA-express.

Loading buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, 5% glycerol. Wash buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP, 5% glycerol.Elution buffer: 50 mM Hepesl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP, 5% glycerol. 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 Loading buffer, 10 volumes of wash buffer, then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

SEC: The peak collected from IMAC was directly applied to a S75 column equilibrated in 50 mM Hepes pH 7.5, 500 m NaCl, 5% glycerol.

Procedure: Akta Express

HiTrap Q: IEX buffer A: 50 mM Hepes, pH 7.5, 10% glycerol. IEX buffer B: 50 mM Hepes, pH 7.5, 1.0 M NaCl.

Protein fraction from desalting column loaded at 1 ml/min, the column was then washed with 10 ml of buffer A and eluted with a 20-minute gradient to 50% buffer B, followed by a step to 100% buffer B.

Concentration: Centricons 10 kDa cut off

## Extraction

**Procedure**
Extraction buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM PMSF, 0.5 mM TCEP. The cell pellets (20 g wet wt) were re-suspended in 50 ml extraction buffer, and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 20,000 rpm in a JA 20 rotor
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**Crystals were grown at 4ºC in 200 nl sitting drops mixing 150 nl of PTPN5 (10 mg/mL in 50mM Hepes pH 7.5, 200mM NaCl ,10mM DTT) with 50 nl of a solution containing 25% PEG3350, 0.2M LiSO4, 100 mM Bis Tris Propane pH 5.5.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**