# HSP90

**PDB:**3OPD

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**
**Entry Clone Source:**
**SGC Clone Accession:**
**Tag:**N-terminal tag: mhhhhhhssgrenlyfqg
**Host:**BL21-(DE3)-V2R-pRare2

## Construct

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

```
MTETFAFQAEINQLMSLIINTFYSNKEIFLRELISNSSDACDKIRYQSLTNQSVLGDEPHLRIRVIPDRVNKTLTVEDSGIGMTKAD
LVNNLGTIARSGTKSFMEALEAGGDMSMIGQFGVGFYSAYLVADRVTVVSKNNEDDAYTWESSAGGTFTVTSTPDCDLKRGTRIVLH
LKEDQQEYLEERRLKDLIKKHSEFIGYDIELMVENTTEK
```

**Vector:**p15-mhl

## Growth

**Medium:**TB
**Antibiotics:**
**Procedure:***Trypanosoma brucei*, N-term domain of HSP90, Tb10.26.1080, was expressed in E. coli BL21(λDE3) V2R pRare2 in TB growth media in the presence of carbenicillin/chloramphenicol (100 µgram/mL and 34 µgram/mL, respectively). A single colony was inoculated into 25 mL of LB with of carbenicillin/chloramphenicol (100 µgram/mL and 34 µgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37°C. Then the culture was transferred into 900 mls of TB with 100 microgram/mL Carbenicillin and 34 microgram/ml chloramphenicol, 0.3 mL of antifoam (Sigma), 9 mls of 0.83 M MgSO4 and trace elements in a 1L bottle and cultured using the LEX system to an $OD_{600}$ of 5, cooled to 15ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15°C.

## Purification

**Procedure**
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and

subsequently onto a 2mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 Â 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. 1 mM TCEP and 1 mM EDTA was added to the eluted Tb10.26.1080.

TEV protease was then added to the protein to cleave the His-tag and the sample was dialysed overnight in 10 mM Hepes, pH 7.5, 500 mM NaCl, 1 mM DTT. The following day, imidazole was added to the sample at 15 mM final concentration, then the sample was loaded onto a 2.5 ml Ni-NTA (Qiagen) column pre-equilibrated with binding buffer + 15 mM imidazole, pH 7.5. The sample was allowed to bind to the nickel resin for 30 minutes after which the flow through containing the the cut protein was collected.

The sample was then loaded onto a superdex 75 gel filtration column. The eluted protein (in 10 mM Hepes, pH 7.5 and 500 mM NaCl) was concentrated using a 15 ml Amicon Ultra centrifugal filter device (Millipore) with a 10 kDa cutoff. PP-HSP90 (Tb10.26.1080) was concentrated to 31.3 mg/ml and stored at 4 μC. The protein was diluted to 12 mg/ml (0.50 mM) prior to crystallization.

## Extraction

**Procedure**
The culture was harvested by centrifugation. Pellets from 2 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 °C were thawed overnight at 4 °C on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5% CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 °C.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**The protein was crystallized at 18 °C in 25% PEG 8000, 0.2 M NaCl, 0.1 M NaCacodylate pH 5.5 using the hanging drop method.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**