# LmHSP90

**PDB:**3HJC

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**
**Entry Clone Source:**
**SGC Clone Accession:**LmjF33.0312:H265-V690:D6
**Tag:**mhhhhhhssgrenlyfqg
**Host:**BL21-(DE3)-V2R-pRare2.

## Construct

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

HKPLWTRDPKDVTKEEYAAFYKAISNDWEDPAATKHFSVEGQLEFRSIMFVPKRAPFDMFEPNKKRNNIKLYVRRVFIMDNCEDLCP
DWLGFVKGVVDSEDLPLNISRENLQQNKILKVIRKNIVKKCLEMFDEVAENKEDYKQFYEQFGKNIKLGIHEDTANRKKLMELLRFY
STESGEEMTTLKDYVTRMKAGQKSIYYITGDSKKKLETSPFIEQARRRGLEVLFMTEPIDEYVMQQVKDFEDKKFACLTKEGVHFEE
SEEEKQQREEEKAACEKLCKTMKEVLGDKVEKVIVSERLSTSPCILVTSEFGWSAHMEQIMRNQALRDSSMAQYMMSKKTMELNPRH
PIIKELRRRVGADENDKAVKDLVFLLFDTSLLTSGFQLEDPTGYAERINRMIKLGLSLDEEEEEAAEAPVAETAPAEV

**Vector:**p15-mhl

## Growth

**Medium:**TB
**Antibiotics:**
**Procedure:**Leishmania major PP-HSP90(LmjF33.0312) was expressed in E. coli BL21(λDE3) V2R pRare2 in TB growth media in the presence of carbenicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL, respectively). A single colony was inoculated into 25 mL of LB with of carbenicillin/chloramphenicol (100 microgram/mL and 34microgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37degC. 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 OD600 of 5, cooled to 15degC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15degC.

## 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 - 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 - 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 PP-HSP90 (LmjF33.0312).

## 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 degC were thawed overnight at 4 degC 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 sonicated for 10 minutes and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.
**Concentration:**The sample was then loaded onto a superdex 200 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 (LmjF33.0312) was concentrated to 28.6 mg/ml and stored at 4C. The protein was diluted to 15 mg/ml before use in crystal trials.
**Ligand**
**MassSpec:**
**Crystallization:**The protein was crystallized in 2.5 M Ammonium sulfate, 0.1 M Sodium acetate pH 4.6 at 20degC in using the sitting drop method.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**