

# Pv-ASL

PDB:2HVG

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**Pv003765

**Entry Clone Source:**Plasmodium vivax Salvador I genomic DNA

**SGC Clone Accession:**

**Tag:**N-terminal histag with thrombin protease cleavage site: mgsshhhhhssglvprgs

**Host:**BL21(DE3)-R3

## Construct

**Prelude:**

**Sequence:**

```
mgsshhhhhssglvprgsEHLKNISPIDGRYKKACGELSAFFSEHALIKHRIIVEVRWLLFLNEEELFFEKVTDHSVEVLNQTATN  
ITDSDIARVKAIEEETNHDVKAVEYFVKEKLNKSKREDLLKIKEYVHYLCTSEDINNVAATCLKACLNDVVIPCLEKIMLKLKDLA  
VEYSHVPLLSRTHGQPASSTTFGKEMANFYARIHHHVGVIIRRVKCAKFNAGVGNFNAHKVASKDTDWNTIGLFLKKHFNLTYSIY  
CTQIQDHDYICELCDGLARANGTLIDLVDIWLWYISNNLLKLVKEKEVGSSTMPHKVNPIDFENAEGNLHIANAFFKLFSSKLPTS  
RLQRDLSDSTVLRNIGSSLAYCLIAYKSVLKGLNKIDIDRRNLEELNQNWSTLAETIQIWMKRHNYVDAYEELKQFTRGKVIDQKI  
MQEFIKTKCAFLPQDVVDQLLELTPATYTYADYLAKNVERLSGE
```

**Vector:**p28a-thrombin-LIC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**Pv-ASL was expressed in E. coli BL21(DE3)-R3 in M9 SeMet High Yield growth media (Medicilon) in the presence of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL, respectively). A single colony was inoculated into 50 mL of LB media with of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) in a 250mL flask and incubated with shaking at 250 rpm overnight at 37 °C. The 50ml over night culture, was centrifuged at 2000 rpm and the cell pellets were washed with M9 SeMet High yield growth media. The resuspended pellet was added to 1.8 L of M9 SeMet High-Yield growth media with 100 microgram/mL ampicillin, 34 microgram/ml chloramphenicol and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 around 1.5 . The inhibitory amino acid cocktail and SeMet was then added to the cultures and the temperature was lowered to 15 °C. Twenty minutes later, the cultures were induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

## Purification

## **Procedure**

Column 1: The cleared cell lysate was loaded onto a DE52(Whatman) column packed with 10 g of resin pre-equilibrated with binding buffer and subsequently onto a 2 mL Ni-NTA column (Qiagen) at approximately 1.5 mL/min. When all the lysate was loaded, 20 mL of Binding Buffer was added to the DE52 column. Then the Ni-NTA column was washed with 200 mL of Wash Buffer at 2 □ 2.5 mL/min. After washing, the protein was eluted from the Ni-NTA column with 15 mL of Elution Buffer. EDTA was added immediately to 1 mM; and DTT was added to 5 mM 15 minutes later.

Column 2: The eluted PV-PFB0295w::H6 was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl. The collected fractions corresponding to the eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore) with a 5 kDa cutoff.

Stock concentration: 18 mg/mL

## **Extraction**

### **Procedure**

The culture was harvested; and the cell pellet from 4 L of culture was suspended in 160 mL of binding buffer with protease inhibitors (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and kept in 4 x 50 mL Falcon tube at ~ 80 °C. Before purification, the cell pellet was thawed overnight at 4 °C. Prior to mechanical lysis, each tube of cell suspension was pretreated with 0.5 % CHAPS and 500 units of benzonase (per 40 mL of resuspended cell pellet) for 40 minutes at room temperature. Then the cells were mechanically lysed in a microfluidizer (Microfluidizer Processor, M-110EH) pre-equilibrated in Binding Buffer at approximately 18000 psi. The lysate was centrifuged at 24,000 rpm for 20 minutes at 10 °C.

### **Concentration:**

### **Ligand**

### **MassSpec:**

**Crystallization:** Purified Pv-ASL was crystallized using the sitting drop vapor diffusion method in a VDX plate (Hampton Research) with 500 µL of mother liquor at 18 °C. 1 µL of the protein solution was mixed with 0.75µL of the reservoir solution containing 1.7 M Ammonium sulfate, 0.2 M Potassium sodium tartrate, and 0.1 M Sodium citrate pH 5.6. Crystals appeared within 3 days.

### **NMR Spectroscopy:**

### **Data Collection:**

### **Data Processing:**