# Pf-UEV1: Plasmodium falicparum homologue of ubiquitin conjugating enzyme E2 variant UEV1

**PDB:**2Q0V

## Revision

**Revision Type:**created
**Revised by:**created
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**Entry Clone Accession:**PFC0255c
**Entry Clone Source:**
**SGC Clone Accession:**PFC0255c:I5-N142; plate MAC02D::D12
**Tag:**N-terminal His6-tag with integrated TEV cleavage site (*): mhhhhhhssgrenlyfq*g
**Host:**E. coli BL21-(DE3)-R3-pRARE2

## Construct

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

gIVPRSFRLLDELERGQKGNVSEGVSFGLESADDITLSNWSCTIFGQPGTVFENRIYSLTIFCDDNYPDSPPTVKFDTKIEMSCVDN
CGRVIKNNLHILKNWNRNYTIETILISLRQEMLSSANKRLPQPNEGEVYSNN

**Vector:**pET15-MHL

## Growth

**Medium:**TB
**Antibiotics:**100 microG/mL ampicillin and 34 microG/mL chloramphenicol
**Procedure:** single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

## 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 2 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1-1.5 mL/min. After the lysate was loaded, the DE52 was further washed with 10 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. EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 2 mM after 15 minutes.

The sample was loaded onto a Sephadex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak corresponding to monomeric protein were pooled and concentrated to 12 mg/mL using a 15 mL Amicon Ultra centrifugal filter device (Millipore) with a cutoff matching the MW of the protein. The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE. The concentrated protein was stored at -80 degC.

## Extraction

**Procedure**
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with protease inhibitor (1 mM benzamidine-HCl 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 6 minutes and the cell lysate was centrifuged using a Beckman JLA-16.250 rotor at ~38,400 x g (16,000 rpms) for 45 minutes at 4 degC.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**The protein was crystallized at 20 degC in 1.4 M Na/KPO4, pH 7.0, 1 mM DTT, with 1 microL buffer added to 1 microL protein using the hanging drop vapour diffusion method.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**