

# UEV1

**PDB:**2A4D

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**gi:40806189

**Entry Clone Source:**MGC

**SGC Clone Accession:**ubc62.230.370; SDC021:E1

**Tag:**N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPRGS.

**Host:**E.coliBL21 (DE3)

## Construct

**Prelude:**

**Sequence:**

MGSSHHHHHHSSGLVPRGSTGVKVPNRNFRLLLEELEGQKGVGDGTVSWGLEDDEMTLTRWTGMIIGPPRTIYENRIYSLKIECGPK  
YPEAPPFVRVFTKINMNGVNSSNGVVDPR AISVLAKWQNSYSIKVVLQELRRLMMSKENMKLPQPPEGQCYSN

**Vector:**p28a-LIC

## Growth

**Medium:**TB

**Antibiotics:**

**Procedure:**UEV1 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37°C to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15°C. The culture was centrifuged and the cell pellets were collected and stored at -80°C.

## Purification

### Procedure

The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4degC. The column was washed with wash buffer A (10mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM  $\beta$ -mercaptoethanol), wash buffer B (same as wash buffer A but containing 0.05% Tween with protease 20) and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM  $\beta$ -mercaptoethanol. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol (DTT) and concentrated by ultrafiltration.

## Extraction

### Procedure

The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM  $\beta$ -mercaptoethanol) inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.

### Concentration:

### Ligand

### MassSpec:

**Crystallization:** Purified UEV1 was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (12 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 32% PEG1500, 0.2 M NaCl, 1 MM DTT, 0.1 M Na Cacodylate at pH 6.5 in 293K temperature.

### NMR Spectroscopy:

### Data Collection:

### Data Processing: