# YWHAE

**PDB:**2BR9

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**gi:5803225
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**N-terminal: His-tag with integrated thrombin protease site:
MHHHHHHSSGVDLGTENLYFQ*SH
**Host:**BL-21(DE3)

## Construct

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

**Vector:**pLIC-SGC

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**10 mL of LB media with 100 µg/mL ampicillin, in 50 mL Falcon tubes, was inncoculated with 5 µl of a glycerol stock of each of the above cultures. The culture was left in a shaker overnight at 37°C and 200 rpm.

1 L of TB media with 100 µg/mL ampicillin was innoculated using the 10mL overnight culture and placed in a shaker at 37°C and 200 rpm.

When the flasks had reached an OD600 ~0.5, IPTG was added to a concentration of 0.75 mM and the temperature was reduced to 18°C.

The cells were spun down (4000 rpm, 15 mins) and the pellets (approx. 25 mLs volume) resuspended in 20 mLs Extraction Buffer. The resuspended cell pellets were placed in the -80°C freezer.

## Purification

**Procedure**
Column 1 : Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

Column 1: Ni-NTA

Wash buffer I (WB1): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 10 mM Imidazole pH 8.0

Wash buffer II (WBII): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 30 mM Imidazole pH 8.0

Elution buffer (EB): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 250 mM Imidazole pH 8.0,

Procedure: Total volume of Ni-NTA added to BioRad drip column: 4 mL (50%) Resin washed with 12.5 ml of WB1. The supernatent was applied to a column using 5 ml pipette and allowed to pass over the resin. The flow through was collected in a 50 mL falcon tube and applied once more to the column. Two wash steps followed. Wash with 12.5 mL of WBI. Wash with 12.5 ml column vols of WBII. Elute with 14 mls of EB into 7x2 mL fractions.

Column 2 : Size exclusion using a S75 16/60 column

Gel Filtration (GF) Buffer: 50 mM Hepes pH 8.0, 150 mM NaCl

Procedure: The column was pre-equilibrated with two column volumes of GF buffer (flow rate 1 mL/min). The fractions from gel filtration that contained YWHAEA were pooled and concentrated to a final colume of 1.5 mL. One gel filtration run at a flow rate of 1 mL/min was sufficient to purify YWHAEA to greater than 95 % as judged by SDS-PAGE gel stained using Coomassie Blue.

## Extraction

**Procedure**
Extraction buffer (EX): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 10 mM Imidazole pH 8.0

All extraction steps were carried out at 4°C. 1 tablet protein inhibitor in 10ml EX buffer was added to the 1L growth pellet. Total vol: 45 mls (estimate).

Cell breakage: 5 passes through the Emulsiflex C5 high pressure homogeniser. Total vol: 50 mL (estimate).

Centrifuge for 30 mins at 16000 rpm and 4°C to remove cell debris.

Discard pellet.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**The purified YWHAE was then concentrated to 11 mg/ml and distributed into 12 x 50 mL aliquots and frozen at -80°C. YWHAE was crystallised in the presence of a phosphorylated peptide. The peptide RRQRpSAP where pS stands for a phosphorylated Serine was synthesised by Thermo Electron Corporation (www.thermo.com). The peptide was dissolved to a concentration of 40 mM in water. Before crystallisation set-up YWHAE was thawed and again concentrated to 34 mg/mL. The peptide and YWHAE were mixed to a molar ratio of 3:1 peptide-to-YWHAE.

Crystals grew from a 1:1 ratio mix of YWHAEA/peptide-to-reservoir (40 % MPD, 5 % PEG 10000, 0.1 M cacodylate pH 6.5).

**NMR Spectroscopy:**

**Data Collection:**

**Data Processing:**