# EBF1

**PDB:**3MQI

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**BC038805
**Entry Clone Source:**Mammalian Gene Collection
**SGC Clone Accession:**
**Tag:**N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
**Host:***E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

## Construct

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

mhhhhhhssgvdlgtenlyfq*smEHATPCIKAISPSEGWTTGGATVIIIGDNFFDGLQVIFGTMLVWSELITPHAIRVQTPPRHIP
GVVEVTLSYKSKQFCKGTPGRFIYTALNEPT

**Vector:**pNIC-Bsa4

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 100 µl PPG P2,000 81380 anti-foam solution (Fluka). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until $OD_{600}$ reached ~2. The culture was down-tempered to 18 °C over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x *g*, 10 min, 4 °C). The resulting cell pellet (32 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

## Purification

**Procedure**
**Columns**

IMAC: 2 x Ni-charged 1 ml HiTrap Chelating HP (GE Healthcare)Gel filtration column: HiLoad 16/60 Superdex 200 Prep Grade (GE Healthcare)

**Procedure**

Purification of the protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using an Vivaspin 20 centrifugal filter device, 10,000 NMWL (Sartorius) to a concentration of 5.2 mg/ml in a volume of 0.7 ml . The identity of the protein was confirmed by mass spectrometry.

**Tag removal**

The N-terminal histidine tag was proteolytically removed by incubating the target protein with His-tagged TEV protease in a molar ratio of 70:1 at room temperature for three hours. The proteolytic reaction went to completion, as judged by SDS-PAGE. Target protein was purified from tag and protease by passing the reaction mixture over a Ni-charged 1 ml HiTrap Chelating HP column (GE Healthcare) pre-equilibrated with a buffer containing 20 mM HEPES, 500 mM NaCl, 10 % glycerol , pH 7.5, 50 mM imidazole, 0.5 mM TCEP. The protein was retrieved from flow through.The protein was concentrated and the buffer was changed to GF buffer with 2 mM TCEP using a Vivaspin 20 centrifugal filter device with 5,000 MWCO (Sartorius). The final protein concentration was determined to 6.9 mg/ml in a volume of 0.33 ml.

## Extraction

**Procedure**
The cell suspension was quickly thawed in water. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.2 µl protein solution (6.9 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.1 M Tris pH 9, 0.3 M trimethylamine n-oxide and 23% PEG monomethyl ether 2000. The plate was incubated at 4 ºC and crystals appeared within 5 days. The crystals were soaked for 24 h in EMTS and were quickly transferred to cryo solution containing well solution (0.3 M trimethylamine n-oxide, 0.1 M tris pH 9, 23% PEG monomethyl ether 2000) complemented with 20% glycerol, and flash frozen in liquid nitrogen.and flash frozen in liquid nitrogen.
**NMR Spectroscopy:**
**Data Collection:**Diffraction data to 2.3 Å resolution was collected at the Bessy beamline BL 14-1. he crystal belonged to space group C 1 2 1 with cell parameters of a=86.29 b=57.07 Å and c=69.14 Å. β=93.17
**Data Processing:**Data was integrated with XDS and scaled with XSCALE. Phases were

calculated with SOLVE. RESOLVE was used to build the initial model. The asymmetric unit consists of three molecules. The model was then improved by manual editing in COOT and refinement in REFMAC5 from the CCP4 suite.