

# BRDT

**PDB:**2RFJ

## Revision

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**Entry Clone Accession:**gi|46399198

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**Tag:**mhahhhhhsssgvdlgtenlyfq\*s(m) TEV-cleavable (\*) N-terminal his6 tag.

**Host:**BL21(DE3)-R3-pRARE2

## Construct

**Prelude:**

**Sequence:**

mhahhhhhsssgvdlgtenlyfq\*smNTKKNGRLTNQLQYLQKVVLKDLWKHSFSWPFQRPVDAVKLQLPDYYTIIKNPMDLNTIKKRL  
ENKYYAKASECIEDFNTMFNSNCYLYNKGDDIVLMAQALEKLFMQMQLSQMPQEE

**Vector:** pNIC28-Bsa4.

## Growth

**Medium:**

**Antibiotics:**

**Procedure:** 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 liter of TB media containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD600 reached ~2.0. After that the temperature was adjusted to 18°C. Expression was induced overnight using 1mM IPTG. The cells were collected by centrifugation and the pellets were frozen.

## Purification

**Procedure**

Column 1: Ni-affinity chromatography ( HisTrap FF crude, 5 ml )All purification steps were carried out using an AKTAexpress system (GE Healthcare) at 7°C. The lysate was loaded on a pre-equilibrated His-trap column at 0.8 ml/min, using a standard purification method. After loading, the column was washed at 0.8 ml/min with 10 ml binding buffer, then 20 ml wash buffer, and protein was eluted with 5 ml of elution buffer. The peak fraction was collected automatically according to A280.Column 2: Size exclusion chromatography (HiLoad 16/60 Superdex S75)The BRDTA containing fraction eluted of the Ni-affinity chromatography was automatically loaded on the SEC column at 1.2 ml/min. BRDTA eluted at a retention time corresponding to the

monomeric protein. Eluted fractions were 95% pure as judged by SDS-PAGE, and confirmed by mass spectrometer (expected mass: 16614 Da). Tag cleavage and column 3: The protein was incubated with TEV protease overnight at 4°C. After cleavage, the protein was passed through a Ni-sepharose column (0.5 ml bed volume) to capture the cleaved tag and other contaminants. The molecular mass after tag cleavage was confirmed by mass spectrometry (14149 Da).

## Extraction

### Procedure

The cell pellet (35 g) from 2 L culture was re-suspended in one volume (35 ml) of 2x extraction buffer. The re-suspended cells were lysed by one passage through a high-pressure cell breaker (Constant Systems) and subsequent sonication; the cell breaker was washed with 1x extraction buffer, bringing the total volume to 120 ml. DNA was precipitation by addition of PEI (polyethyleneimine, pH 7.5) to a final concentration of 0.15 % during an incubation time of 30 min on ice, followed by a centrifugation at 17,000 rpm (4°C); The supernatant was further cleared by filtration through a 0.2 µm filter (serum Acrodisc).

**Concentration:** The protein was concentrated to 35 mg/ml using a Centricon device (5 kDa cut off).

### Ligand

### MassSpec:

**Crystallization:** Crystals were obtained using the vapor diffusion method. Drops were setup in 96 well sitting drop plates by mixing 100nl of the concentrated protein (35 mg/ml) with 100nl of a well solution containing 0.20M Na/K(tart); 20.0% PEG 3350; 10.0% EtGly.

### NMR Spectroscopy:

**Data Collection:** Diffraction data were collected at 2.0 Å resolution from a crystal that was cryo-protected using 20% ethylene glycol (end concentration) at the SLS beam-line SAX10.

### Data Processing: