

# Molecular Biology

**Entry Clone Accession:** IMAGE:6149862

**Entry Clone Source:** MGC

**SGC Construct ID:** BRD7A-c005

**Protein Region:** E129-S239

**Vector:** pNIC28-Bsa4

**Tag:** N-terminus His<sub>6</sub> tag followed by a TEV cleavage site sequence

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

**Sequence (with tag(s)):**

MHHHHHHSSGVDLGTENLYFQSMEVEQTPLQEALNQLMRQLQRKDPSAFFSFPVTDFI  
APGYSMIKHPMDSTMKEKIKNNDYQSIEELKDNFKLMCTNAMIYNKPETIYYKAACK  
LLHSGMKILSQERIQS

**Sequence after tag cleavage:**

SMEEVEQTPLQEALNQLMRQLQRKDPSAFFSFPVTDFIAPGYSMIKHPMDSTMKEKIK  
NNDYQSIEELKDNFKLMCTNAMIYNKPETIYYKAACKLLHSGMKILSQERIQS

**DNA Sequence:**

CATATGCACCATCATCATCATTCTCTGGTAGATCTGGTACCGAGAACCTGTA  
CTTCCAATCCATGGAAGAAGTAGAACAGACACCCCTCAAGAACGCTTGAAATCAACT  
GATGAGACAATTGCAGAGAAAAGATCCAAGTGCTTCTTCATTCCCTGTGACTGAT  
TTTATTGCTCCTGGCTACTCCATGATCATTAAACACCCAATGGATTAGTACCATGAAA  
GAAAAGATCAAGAACAAATGACTATCAGTCCATAGAAGAACTAAAGGATAACTCAA  
CTAATGTGTACTAATGCCATGATTACAATAAACAGAGACCATTATTATAAGCTGC  
AAAGAAGCTGTTGCACTCAGGAATGAAAATTCTAGCCAGGAAAGAATTAGAGCTG  
ACAGTAAAGGTGGATACGGATCCGAA

## Protein Expression

**Medium:** TB media complemented with 50 µg/mL of kanamycin and 34 µg/mL of chloramphenicol.

**Antibiotics:** Kanamycin and chloramphenicol.

**Procedure:** Each 1L culture was inoculated with 10 mL of a starter culture grown at 37°C overnight. Then, expression was induced with 0.15 mM IPTG when the OD<sub>600nm</sub> reached 1.2, and incubated at 18°C overnight.

## Protein Purification

**Procedure:** After expression, cultures were centrifuged at 3,500 g and pellets were frozen with liquid nitrogen. 10 g of bacterial pellet were resuspended in 100 mL of buffer A (50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM imidazole, 5% glycerol, 0.5 mM TCEP) containing cOmplete EDTA-free protease inhibitors from Roche. Cell disruption was carried out using an Avestin EmulsiFlex-c5 homogenizer. Lysates were centrifuged at 35,000 g for 1h at 4°C. Samples were incubated with 2 mL of Ni-Sepharose 6 FF 1h at 4°C. After removing the non-bound lysate, beads were washed with buffer A followed by an extra wash with buffer A with 50 mM imidazole. Elution was achieved with buffer A containing 150 mM imidazole. Samples were then dialyzed into buffer A at 4°C overnight, in the presence of TEV protease. To keep the untagged BRD7, a second affinity chromatography using Ni-Sepharose 6 FF was performed. The flow through was then concentrated with an Amicon 3K centrifugal filter, and applied to a size exclusion chromatography column HiLoad 16/600 superdex 75 pg in a buffer containing 10 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol and 0.5 mM TCEP. The peak corresponding with

BRD7 was concentrated to 4.24 mg/mL. Purity and protein identity were confirmed using SDS-PAGE, Q-TOF mass spectrometry, and tryptic digestion. The final yield was 2.2 mg of protein per 1 L of culture.

**Columns:** Column 1: 2 mL Ni-NTA; Column 2: 2 mL Ni-NTA; Column 3: HiLoad 16/600 Superdex 75

**Concentration:** 4.24 mg/ml

**Mass-spec Verification:** Yes

## Structure Determination

**Crystallization:** Prior crystallization, BRD7 was incubated with the compound BI9864 (1:3 protein:compound molar ratio) for 1h at 4°C. Crystal plates were incubated at 4°C for 5 days. Crystals grew in a condition containing 20% PEG6000, 10% ethylene glycol, 0.1 M HEPES pH 7.0, 0.01 M ZnCl<sub>2</sub>. Crystals were cryo-protected with 20% ethylene glycol, and frozen in liquid nitrogen.

**Data Collection:** Diffraction data were collected at Diamond Light Source beamline I04-1 using a Pilatus 6M-F detector.

**Data Processing:** Data were indexed and integrated using XDSgui, and scaled and analyzed with AIMLESS from the CCP4i suite. The structure was solved using Phaser. The search model was an internal BRD9 structure, which shares a 37.6% sequence homology with BRD7. The resolution obtained was 1.5 Å. Refinement was carried out using REFMAC, and restraints for BI9864 were obtained with PRODUG.