

Clone sample ID: RBBP7:JMC01G-E12:C204609

Construct ID: RBBP7-11

Vector: pFBOH-LIC

N-terminal tag: mgsshhhhhssglvprgs **C-terminal tag:** N/A

Residues (from-to): 1- 425

mgsshhhhhssglvprgsMASKEMFEDTVEERVINEEYKIWKKNTPFLYDLVMTHALQWPSLTVQW
LPEVTKPEGKDYALHWLVLTHTSDEQNHLVVARVHIPNDDAQFDASHCDSKGEFGGFGSV
TGKIECEIKINHEGEVNRARYMPQNPHEIATKTPSSDVLVFDYTKHPAKPDPSGECNPDLRLRGH
QKEGYGLSWNSNLSGHLLSASDDHTVCLWDINAGPKEGKIVDAKAIFTGHSVAVVEDVAWHLLH
ESLFGSVADDQKLMIWDRSNTTSKPSHLVDAHTAEVNCLSFNPYSEFILATGSADKTVALWDL
RNLKLLHTFESHKDEIFQVHWSPHNETILASSGTDRLNVWDLISKIGEEQSAEDAEDGPPELL
FIHGGHTAKISDFSWNPNPWWVICSVSEDNIMQIWQMAENIYNDEESDVTTSELEGQGS

Host: *sf9*

Yield: 0.5 - 1 mg/L

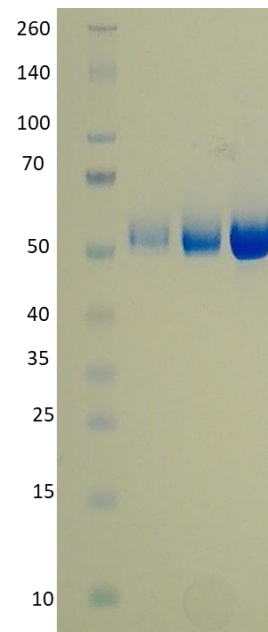
Storage buffer: 20 mM Tris, pH 8.0, 200 mM NaCl, 5% glycerol, 1 mM TCEP

Purity uncut protein: >90 %

Purity assessment: SDS-PAGE on 4-12% Bis-Tris Gel (Life technology)

Lane 1: Spectra Multicolour Broad Range Protein Ladder,
Thermo Fisher Scientific

Lanes 2- 4: 1, 2 and 5 µg after Gel filtration



Construct and Expression:

DNA fragment encoding RBBP7 (1- 425) was cloned into pFBOH-LIC donor plasmid. The resulting plasmid was transformed into DH10Bac Competent E. coli cells (Invitrogen) and a recombinant Bacmid DNA was purified and followed by a recombinant baculovirus generation in Sf9 insect cells. Sf9 cells grown in HyQ® SFX insect serum-free medium (Thermo Scientific) were infected with 10 ml of P3 viral stocks per 1 L of suspension cell culture and incubated at 27°C using a platform shaker set at 150 rpm. The cells were harvested by centrifugation (4000 rpm for 10 mins at 4°C) after 72 hours of post infection time, when viability dropped to 70-80%.

Harvest and cell lysis:

Harvested cells were re-suspended in 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5 mM imidazole and 5% glycerol, 1X protease inhibitor cocktail (100 X protease inhibitor stock in 70% ethanol (0.25 mg/ml Aprotinin, 0.25 mg/ml Leupeptin, 0.25 mg/ml Pepstatin A and 0.25 mg/ml E-64) or Pierce™ Protease Inhibitor Mini Tablets, EDTA-free. The cells were lysed chemically by rotating for 30 min after addition of 1 mM PMSF, 1 mM TCEP, 0.5% NP40 and 15 µL Benzonase Nuclease (In-House) followed by sonication at frequency of 7 (5" on/7" off) for 2 min (Sonicator 3000, Misoni). The crude extract was clarified by high-speed centrifugation (60 min at 36,000 xg at 4°C) by Beckman Coulter centrifuge.

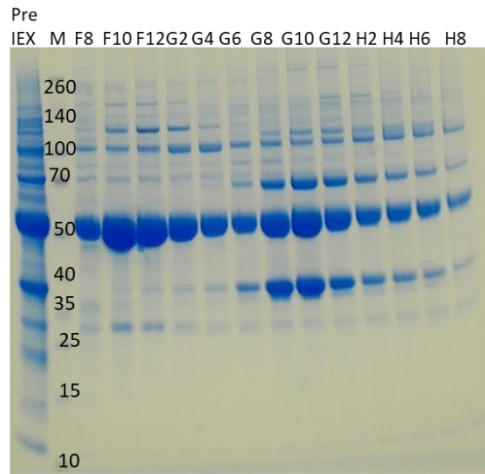
Purification columns:

- Ni-NTA
- Anion Exchange Resource Q
- HiLoad Superdex75 16/600 * other gel filtration columns have been used as well

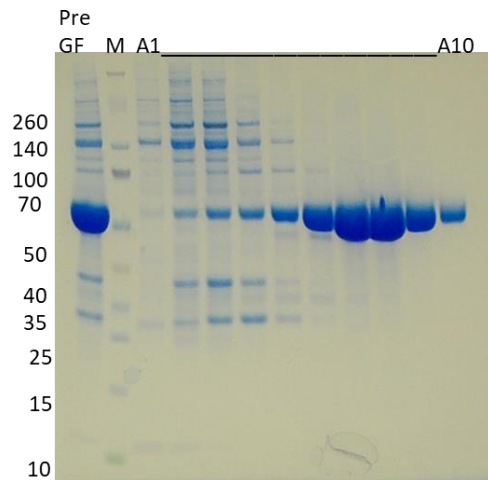
Purification SOP:

Clarified lysate was then incubated for ~one hour at 4°C with pre-equilibrated Ni-NTA (Qiagen) in batch adsorption format on a rotating platform. Resin was then transferred to an open gravity column (Bio-Rad) and was washed with binding buffer followed by washing buffer containing 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole and 30mM Imidazole, respectively. Protein was then eluted with 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, 250 mM imidazole. Next, the eluate was diluted 10x with Buffer A (20 mM Tris, pH 8.0) and loaded onto a Resource Q ion exchange column (GE Healthcare) and eluted over 40 CV with Buffer B (20 mM Tris, pH 8.0, 1 M NaCl. After analysis by SDS- PAGE, fractions from the first peak containing the protein were pooled and 1 mM TCEP was added before concentrating. To further purify the protein, it was loaded on a gel filtration HiLoad Superdex 75 16/60 (GE Healthcare) column pre-equilibrated in 20 mM Tris, pH 8.0, 200 mM NaCl, 5% glycerol, 1 mM TCEP. Fractions were assessed by SDS-PAGE electrophoresis and those containing pure protein (second peak) were pooled, concentrated and flash frozen for storage at -80°C.

Ion Exchange Chromatography: 6ml Resource Q



Gel Filtration Chromatography: Superdex75 16/600



Expected size: 49846.27 Da

Mass Spec: 49759.21 Da