Clone sample ID: TEAD4:MVC007-E02:C238480

Construct ID: TEAD4:P212-434-his

Vector: pET28-MHL

N-terminal tag: MHHHHHHSSGRENLYFQG

Residues (from-to): 212-434

MHHHHHHSSGRENLYFQGPPWQGRSVASSKLWMLEFSAFLEQQQDPDTYNKHLFVHIGQSS
PSYSDPYLEAVDIRQIYDKFPEKKGGLKDLFERGPSNAFFLVKFWADLNTNIEDEGSSFYGVSS
QYESPENMIITCSTKVCSFGKQVVEKVETEYARYENGHYSYRIHRSPLCEYMINFIHKLKHLPEK
YMMNSVLENFTILQVVTNRDTQETLLCIAYVFEVSASEHGAQHHIYRLVKE

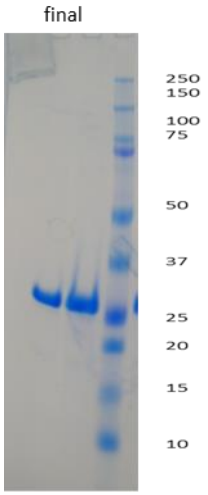
Host: E. coli BL21 (DE3) –Codon plus

Purity uncut protein: >95%

Yield: 4 mg/L

Storage buffer: 20 mM Tris pH 8.0, 150 mM NaCl and 10 mM 2-Mercaptaethanol

SDS-PAGE gel on 4-12% Bis-Tris Gel (Life technology) with Precision Plus Protein standard, Bio-Rad:



Construct and Expression

DNA fragment encoding TEAD4 (residues 212-434) was amplified by PCR and sub-cloned into the pET28-MHL vector downstream of the poly-histidine coding region. Following transformation into E. coli BL21 (DE3) –Codon plus, the cells were amplified at 37°C by inoculating Terrific Broth with overnight culture, both supplemented with 50 µg/ml Kanamycin and 35 µg/ml chloramphenicol. When the OD600 of the culture reached 0.8- 1.5, the temperature was lowered to 18°C and the target protein was over-expressed by inducing cells with 0.5 mM IPTG (isopropyl-1-thio-D-galactopyranoside) and incubated overnight before being harvested (7000 rpm for 10 minutes at 4˚C) using a Beckman Coulter centrifuge.

Harvest and cell lysis

Harvested cells were re-suspended in 20 mM Tris-HCl pH 7.5, containing 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 Roche complete EDTA-free protease inhibitor cocktail tablet and 10 mM 2-Mercaptaethanol. The cells were lysed chemically by rotating 30 min with CHAPS (final concentration of 0.5%) and 5 µl/L Benzonase nuclease (in house) followed by sonication at frequency of 8.0 (10” on/10” off) for 4 min (Sonicator 3000, Misoni). The crude extract was clarified by high-speed centrifugation (60 min at 36,000 ×g at 4˚C) by Beckman Coulter Centrifuge.

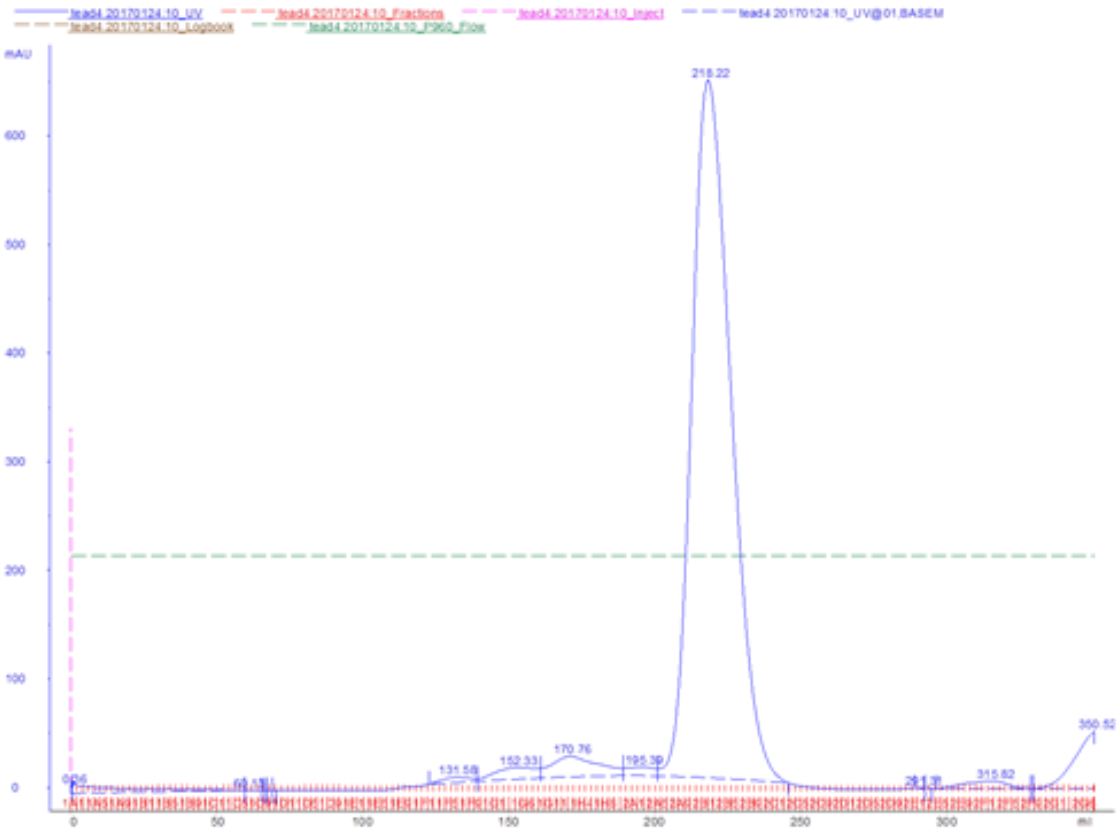
Purification

Purification columns:

- Ni-NTA affinity resin
- Superdex75 26/600

The clarified lysate was then loaded onto an open column containing pre-equilibrated Ni-NTA (Qiagen). The column was washed and eluted by running 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol and 10 mM 2-Mercaptaethanol, containing 20 mM and 250 mM imidazole, respectively. The eluent was further purified by gel filtration on a Superdex75 26/600 using an ÄKTA FPLC (GE Healthcare) pre-equilibrated with 20 mM Tris-HCl pH 8.0, 150 mM NaCl and 10 mM 2-Mercaptaethanol. The purity of the fractions was confirmed on SDS-PAGE gels and the pure fractions were pooled, concentrated and flash frozen.

Superdex75 26/600 gel filtration :



Expected size: 28171.6 Da

Observed Mass: 28172.26 Da