

Molecular Biology

Entry Clone Accession: NM_017545

Entry Clone Source: Origene

SGC Construct ID: HAO1A-c002

Protein Region: M1-S368

Vector: pNIC28-Bsa4

Tag: N-6HIS; TEV cleavage site

Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):

MHHHHHHSSGVDLGTENLYFQSMLPRLICINDYEQHAKSVLPKSIYDYYRSGANDEETL
ADNIAAFSRWKLYPRMLRNVAETDLSTSVLGQRVSMPCV GATAMQRM AHVDGELATV
RACQSLGTGMMLSSWATSSIEEVAEAGPEALRWLQLYIYKDREVTKKLVRQAEKMGYK
AIFVTVDTPYLG NRLLDDVRNRFKLPPQLRMKNFETSTLSFSPEENFGDDSGLAAYVAKAI
DPSISWEDIKWLRRLTSLPIVAKGILRGDDAREAVKHGLNGILVSNHGARQLDGV PATIDV
LPEIVEAVEGKVEVFLDGGVRKGT DVLKALALGAKAVFVGRPIVWGLAFQGEKGVQDV
LEILKEEFRLAMALSGCQNVKVIDKTLVRKNPLAVS

Sequence after tag cleavage:

SMLPRLICINDYEQHAKSVLPKSIYDYYRSGANDEETLADNIAAFSRWKLYPRMLRNVAE
TDLSTSVLGQRVSMPCV GATAMQRM AHVDGELATVRACQSLGTGMMLSSWATSSIEEV
AEAGPEALRWLQLYIYKDREVTKKLVRQAEKMGYKAIFVTVDTPYLG NRLLDDVRNRFK
LPPQLRMKNFETSTLSFSPEENFGDDSGLAAYVAKAIDPSISWEDIKWLRRLTSLPIVAKGI
LRGDDAREAVKHGLNGILVSNHGARQLDGV PATIDVLP EIVEAVEGKVEVFLDGGVRKG
TDVLKALALGAKAVFVGRPIVWGLAFQGEKGVQDVLEILKEEFRLAMALSGCQNVKVI
DKTLVRKNPLAVS

DNA sequence:

CATATGCACCATCATCATCATCATCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGCTCCCCCGGCTAATTTGTATCAATGATTATGAACAACATGCTAAATC
AGTACTTCCAAAGTCTATATATGACTATTACAGGTCTGGGGCAAATGATGAAGAACTT
TGGCTGATAATATTGCAGCATTTCAGATGGAAGCTGTATCCAAGGATGCTCCGGAAT
GTTGCTGAAACAGATCTGTCGACTTCTGTTTTAGGACAGAGGGTCAGCATGCCAATAT
GTGTGGGGGCTACGGCCATGCAGCGCATGGCTCATGTGGACGGCGAGCTTGCCACTG
TGAGAGCCTGTCAGTCCCTGGGAACGGGCATGATGTTGAGTTCCTGGGCCACCTCCTC
AATTGAAGAAGTGGCGGAAGCTGGTCCTGAGGCACTTCGTTGGCTGCAACTGTATATC
TACAAGGACCGAGAAGTCACCAAGAAGCTAGTGCGGCAGGCAGAGAAGATGGGCTA
CAAGGCCATATTTGTGACAGTGGACACACCTTACCTGGGCAACCGTCTGGATGATGTG
CGTAACAGATTCAAACCTGCCGCCACAACCTCAGGATGAAAAATTTGAAACCAGTACTT
TATCATTTTCTCCTGAGGAAAATTTTGGAGACGACAGTGGACTTGCTGCATATGTGGCT
AAAGCAATAGACCCATCTATCAGCTGGGAAGATATCAAATGGCTGAGAAGACTGACAT
CATTGCCAATTGTTGCAAAGGGCATTTCGAGAGGTGATGATGCCAGGGAGGCTGTAA
ACATGGCTTGAATGGGATCTTGGTGTGCAATCATGGGGCTCGACAACCTCGATGGGGTG
CCAGCCACTATTGATGTTCTGCCAGAAATTGTGGAGGCTGTGGAAGGGAAGGTGGAA
GTCTTCCTGGACGGGGGTGTGCGGAAAGGCACTGATGTTCTGAAAGCTCTGGCTCTT
GGCGCCAAGGCTGTGTTTGTGGGGAGACCAATCGTTTGGGGCTTAGCTTTCCAGGGG
GAGAAAGGTGTTCAAGATGTCCTCGAGATACTAAAGGAAGAATTCCGGTTGGCCATG
GCTCTGAGTGGGTGCCAGAATGTGAAAGTCATCGACAAGACATTGGTGAGGAAAAAT
CCTTTGGCCGTTTCCTGACAGTAAAGGTGGATACGGATCCGAA

Protein Expression

Medium: Terrific Broth

Antibiotics: Kanamycin

Procedure: An overnight culture (20 mL LB) was used to inoculate 2L auto-induction TB containing 50 µg/ml each of kanamycin and chloramphenicol. Cells were cultured at 37°C for 6 hours followed by 48 h incubation at 18°C.

Protein Purification

Procedure: Cell pellet from 2 L of culture was re-suspended in 150 mL Extraction Buffer, lysed by sonication for 15 minutes, and centrifuged at 37000 x g for 1 hour at 4°C. The clarified cell extract was incubated with 5 mL of Ni-NTA resin pre-equilibrated with lysis buffer before applying to 1.5 x 10 cm column by gravity flow. The column was washed with 10 column volumes of Binding Buffer, 10 column volumes of Wash Buffer, and eluted with 5 x 5 mL Elution Buffer. Fractions containing hHAO1 were loaded onto gel filtration column (Superdex 200 Hiload 16/60) pre-equilibrated with GF buffer. Fractions containing hHAO1 were pooled.

Extraction Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 20 mM imidazole, 0.5 mM TCEP, 5% glycerol, 1:1000 of Merck Protease Cocktail II, 0.5 mg/mL lysozyme and 0.2 µg/mL benzonase.

Binding Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 20 mM imidazole, 0.5 mM TCEP, 5% glycerol.

Washing Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 40 mM imidazole, 0.5 mM TCEP, 5% glycerol.

Elution Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 250 mM imidazole, 0.5 mM TCEP, 5% glycerol.

GF buffer: 500 mM NaCl, 550 mM HEPES pH 7.5, 0.5 mM TCEP, 5% glycerol.

Concentration: 13.7 mg/ml

Mass-spec Verification: Yes

Structure Determination

Crystallization: Crystals were grown by vapour diffusion at 4°C in sitting drops consisting 100 nL protein (13.7 mg/mL) and 50 nL well solution, equilibrated against well solution containing either 20% PEG3350, 0.1 M bis-tris-propane pH 7.5, 10% ethylene glycol, 0.2 M sodium nitrate. Crystal was cryo-protected with 20-25% ethylene glycol before flash-cooling in liquid nitrogen.

Data Collection: *Beamline:* Dmnd I03; *Resolution:* 1.35 Å

Data Processing: hHAO1 structure was solved by molecular replacement with PHASER using [2NZL](#) as the search model, followed by iterative cycles of REFMAC5 including TLS refinement, and manual model building in Coot.