# 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
ADNIAAFSRWKLYPRMLRNVAETDLSTSVLGQRVSMPICVGATAMQRMAHVDGELATV
RACQSLGTGMMLSSWATSSIEEVAEAGPEALRWLQLYIYKDREVTKKLVRQAEKMGYK
AIFVTVDTPYLGNRLDDVRNRFKLPPQLRMKNFETSTLSFSPEENFGDDSGLAAYVAKAI
DPSISWEDIKWLRRLTSLPIVAKGILRGDDAREAVKHGLNGILVSNHGARQLDGVPATIDV
LPEIVEAVEGKVEVFLDGGVRKGTDVLKALALGAKAVFVGRPIVWGLAFQGEKGVQDV
LEILKEEFRLAMALSGCQNVKVIDKTLVRKNPLAVS
**Sequence after tag cleavage:**
SMLPRLICINDYEQHAKSVLPKSIYDYYRSGANDEETLADNIAAFSRWKLYPRMLRNVAE
TDLSTSVLGQRVSMPICVGATAMQRMAHVDGELATVRACQSLGTGMMLSSWATSSIEEV
AEAGPEALRWLQLYIYKDREVTKKLVRQAEKMGYKAIFVTVDTPYLGNRLDDVRNRFK
LPPQLRMKNFETSTLSFSPEENFGDDSGLAAYVAKAIDPSISWEDIKWLRRLTSLPIVAKGI
LRGDDAREAVKHGLNGILVSNHGARQLDGVPATIDVLPEIVEAVEGKVEVFLDGGVRKG
TDVLKALALGAKAVFVGRPIVWGLAFQGEKGVQDVLEILKEEFRLAMALSGCQNVKVI
DKTLVRKNPLAVS
**DNA sequence:**
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGCTCCCCCGGCTAATTTGTATCAATGATTATGAACAACATGCTAAATC
AGTACTTCCAAAGTCTATATATGACTATTACAGGTCTGGGGCAAATGATGAAGAAACTT
TGGCTGATAATATTGCAGCATTTTCCAGATGGAAGCTGTATCCAAGGATGCTCCGGAAT
GTTGCTGAAACAGATCTGTCGACTTCTGTTTTAGGACAGAGGGTCAGCATGCCAATAT
GTGTGGGGGCTACGGCCATGCAGCGCATGGCTCATGTGGACGGCGAGCTTGCCACTG
TGAGAGCCTGTCAGTCCCTGGGAACGGGCATGATGTTGAGTTCCTGGGCCACCTCCTC
AATTGAAGAAGTGGCGGAAGCTGGTCCTGAGGCACTTCGTTGGCTGCAACTGTATATC
TACAAGGACCGAGAAGTCACCAAGAAGCTAGTGCGGCAGGCAGAGAAGATGGGCTA
CAAGGCCATATTTGTGACAGTGGACACACCTTACCTGGGCAACCGTCTGGATGATGTG
CGTAACAGATTCAAACTGCCGCCACAACTCAGGATGAAAAATTTTGAAACCAGTACTT
TATCATTTTCTCCTGAGGAAAATTTTGGAGACGACAGTGGACTTGCTGCATATGTGGCT
AAAGCAATAGACCCATCTATCAGCTGGGAAGATATCAAATGGCTGAGAAGACTGACAT
CATTGCCAATTGTTGCAAAGGGCATTTTGAGAGGTGATGATGCCAGGGAGGCTGTTAA
ACATGGCTTGAATGGGATCTTGGTGTCGAATCATGGGGCTCGACAACTCGATGGGGTG
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 from hHAO1 protein pre-incubated with 2 mM CCPST, equilibrated against well solution containing 30% PEG1000, sodium malonate-imidazole-boric acid pH 8.0. Crystal was cryo-protected with 20-25% ethylene glycol before flash-cooling in liquid nitrogen.

**Data Collection:** *Beamline:* Dmnd I03; *Resolution:* 1.2 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.