# MAT2A

**PDB:**2P02

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**MAT 2AA-s001 (gi:5174529 )
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**N-terminal His-tag with TEV protease cleavage site: (Tag sequence in lowercase):
**Host:**E.coli strain Rosetta

## Construct

**Prelude:**
**Sequence:**

mhhhhhhssgvdlgtenlyfq*sMNGQLN GFHEAFIEEGTFLFTSESVGEGHPDKICD QISDAVLDAHLQQDPDAKVACETVAKT
GM ILLAGEITSRAAVDYQKVVREAVKHIGYD DSSKGFDYKTCNVLVALEQQSPDIAQGVH LDRNEEDIGAGDQGLMFGYATDET
EECMP LTIVLAHKLNAKLAELRRNGTLPWLRPDS KTQVTVQYMQDRGAVLPIRVHTIVISVQH DEEVCLDEMRDALKEKVIKAV
VPAKYLDE DTIYHLQPSGRFVIGGPQGDAGLTGRKII VDTYGGWGAHGGGAFSGKDYTKVDRSAAY AARWVAKSLVKGGLCRRV
LVQVSYAIGVS HPLSISIFHYGTSQKSERELLEIVKKNFD LRPGVIVRDLDLKKPIYQRTAAYGHFGRD SFPWEVPKKLKY

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**10 µl of a glycerol stock was inoculated into 5ml of LB medium (supplemented with Kanamycin, 50µg/ml, chloramphenicol, 34µg/ml) in a 15 ml culture tube and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 1 ml of o/n culture was used to inoculate 1 litre of LB medium and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD600of 1.35. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and cultivated for 16 hrs. Cells were harvested, centrifuged at 6500 rpm for 10 min, and the pellet was stored at -20°C until further use.

## Purification

**Procedure**
Column 1 : 1 ml HisTrap crude (GE/Amersham)
The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of

wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : SuperDex 200 16/60 HiLoad (GE/Amersham)
The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration: 19 mg/ml using Vivaspin 10K concentrators

Enzymatic treatment : His-tag was cleaved by using TEV protease

## Extraction

**Procedure**

**Concentration:**
**Ligand**
**MassSpec:**The mass determined for His-tag cleaved MAT 2AA-c001 is 43754.61 Da. There is a mass difference of 7 Da between the determined and the predicted mass value of cleaved MAT 2AA-c001, which is 43747.8 Da.
**Crystallization:**Prior to crystallization 5 mM S-adenosyl methionine was added to the protein. Crystals were grown by vapor diffusion at 20°C. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 200 mM LiCl, 100 mM TRIS pH 8.0, 20 % PEG 6K, 10 % ethylene glycol. The crystal was transferred to well solution supplemented with 20 % ethylene glycol before flash-cooling in liquid nitrogen.
**NMR Spectroscopy:**
**Data Collection:**Resolution: 1.03 Å; X-ray source: Synchrotron SLS -X10.
**Data Processing:**