MAT1 - Human methionine adenosyltransferase I

PDB:2OBV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MAT1A-s001 (gi|4557737)
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with TEV protease cleavage site (Tag sequence in lowercase)
Host: Rosetta-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMGVFMFT SESVGEGHPDKICDQISDAVLDAHLKQDP NAKVACETVCKTGMVLLCGEITSMAMV
DY QRVVRDTIKHIGYDDSAKGFDFKTCNVLV ALEQQSPDIAQCVHLDRNEEDVGAGDQGL MFGYATDETEECMPLTIILAHKLN
ARMAD LRRSGLLPWLRPDSKTQVTVQYMQDNGAV IPVRIHTIVISVQHNEDITLEEMRRALKE QVIRAVVPAKYLDEDTVYHLQ
PSGRFVIG GPQGDAGVTGRKIIVDTYGGWGAHGGGAF SGKDYTKVDRSAAYAARWVAKSLVKAGLC RRVLVQVSYAIGVAEPLS
ISIFTYGTSQK TERELLDVVHKNFDLRPGVIVRDLDLKKP IYQKTACYGHFGRSEFPWEVPRKLVF

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:10 microL of a glycerol stock was inoculated into 100ml of TB medium (supplemented with 50µg/ml kanamycin) and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 12 ml of o/n culture was used to inoculate 1 litre of TB medium (4x) and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD600 of 1.0. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20°C until further use.

Purification

Procedure
Column 1 : Ni-Sepharose 6 Fast Flow
Procedure: The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the

column was washed with 20 ml of binding buffer and then 20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer.

Column 2 : SuperDex 200 16/60 HiLoad (GE/Amersham)
Procedure: The eluted protein from the Ni-affinity column was loaded on the gel filtration column in GF buffer at 1.0 ml/min on an AKTA Purifier system. Eluted proteins were collected in 2 ml fractions.

Column 3: HP Q column (ion exchange).
Procedure: The MAT1A-k003 was applied to 5ml HP Q column in buffer A and eluted from the column by a linear gradient with buffer B.

Concentration : 16.04 mg/ml using Vivaspin 10K concentrators

Extraction

Procedure
Complete® protease inhibitors (Roche, 1 tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes .
Concentration:
Ligand
MassSpec: Corresponds to theoretical mass, as determined by ESI-TOF MS .
Crystallization: Crystals were grown by vapor diffusion at 20°C. Prior to crystallization 5 mM S-adenosyl methionine was added to the protein. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 200 mM NaF, 20 % PEG 3350, 10 % ethylene glycol.
NMR Spectroscopy:
Data Collection: Resolution: 1.9Å; X-ray source: Rotating anode, Rigaku FR-E superbright .
Data Processing: