ASMTL

PDB:2P5X

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004183
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSLLHKRVVLASASPRRQEILSNAGLRFEVVPSKFKEKLDKASFATPYGYAMETAKQKALEVANRLYQKD
LRAPDVVIGADTIVTVGGLILEKPVDKQDAYRMLSRLSGREHSVFTGVAIVHCSSKDHQLDTRVSEFYEETKVKFSELSEELLWEYV
HSGEPMDKAGGYGIQALGGMLVESVHGDFLNVVGFPLNHFCKQLVKLYYPPRPEDLRRSVKHDSIPAADTFEDLS

Vector:pET28a-LIC

Growth

Medium: Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin
Antibiotics:
Procedure: ASMTL Maf domain was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 microG/ml of kanamycin at 37degC to an OD600 of 1.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15degC

Purification

Procedure
The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 500 mM Ammonium Acetate and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 500 mM Ammonium Acetate, 250 mM imidazole). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 500 mM Ammonium Acetate, at flow rate 4 ml/min. Purification yield was 1.2 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 degC. For the purification the cell paste was thawed and resuspended in lysis buffer (PBS, 0.25 M NaCl, 5 mM imidazol, 2 mM &szilg;-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi
Concentration:5.7 mg/ml
Ligand
MassSpec:expected MW is 27807.6 Da, measured MW is 27676.9279 Da.
Crystallization: Purified ASMTL Maf domain was crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 15% PEG 3350, 0.1 M succinic acid, pH 7.0.
NMR Spectroscopy:
Data Collection:
Data Processing: