HTATIP

PDB:2OU2

Revision

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Entry Clone Accession:GI:36287060
Entry Clone Source:MGC
SGC Clone Accession:HTATIP_05:9A11APC005_9
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gsTRMKNIECIELGRHRLKPWYFSPYPQELTTLPVLYLCEFCLKYGRSLKCLQRHLTKCDLRHPPGNEIYRKGTISFFEIDGRKNKS
YSQNLCLLAKCFLDHKTLYYDTDPFLFYVMTEYDCKGFHIVGYFSKEKESTEDYNVACILTLPPYQRRGYGKLLIEFSYELSKVEGK
TGTPEKPLSDLGLLSYRSYWSQTILEILMGLKSESGERPQITINEISEITSIKKEDVISTLQYLNLINYYKGQYILTLSEDIVDGHE
RAMLKRLLRIDSKCLHFTPKD

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: The protein was expressed in E.coli BL21 (DE3) codon plus RIL in 2L of Terrific Broth (TB) in the presence of 50 microG/ml of kanamycin. Cell were grown at 37degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, incubated overnight at 15degC.

Purification

The crude extract was cleared by centrifugation. The lysate was loaded onto 10 ml Chelating Sepharose column (Amersham Biosciences), charged with Ni 2+ . The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4, containing 0.5 M NaCl , 50 mM imidazole, 5% glycerol and 0.1% CHAPS, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 0.5 M

NaCl, 250 mM imidazole, 5% glycerol, 0.1 % CHAPS). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM HEPES buffer, pH 7.4, and 0.5 M NaCl, at flow rate 4 ml/min. The fractions containing HTATIP were pooled and subjected to treatment of thrombin (Sigma) to remove the His-tag. The protein was further purified to homogeneity on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer containing 20 mM Na Citrate pH 6.0 and eluted with linear gradient of NaCl up to 1 M concentration (20CV). Purification yield was 1.2 mg of theprotein per 1L of culture

Extraction

Cells were harvested by centrifugation. The cell pellets were frozen in liquid nitrogen and stored at -80degC. For the purification, the cell paste was thawed and resuspended in 100ml of lysis buffer with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:5 mg/ml
Ligand
MassSpec: The expected mass for HTATIP is 32997.1 Da, measured mass is 33097.2078 Da.
Crystallization: Purified HTATIP was complexed with acetylcoenzyme A (AcCoA, Sigma) at 1:10 molar ratio of protein:AcCoA and crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 2µl of the protein solution with 1 µl of the reservoir solution containing 16% PEG3350, 0.2 M ammonium acetate, 0.1 M Bis-Tris, pH 6.6.
NMR Spectroscopy:
Data Collection:
Data Processing: