HAT1

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Revision

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Entry Clone Accession:GI:4504341
Entry Clone Source:MGC
SGC Clone Accession:HAT1_05:APC038-C3
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host: E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

gsKKLAEYKCNTNTAIELKLVRFPEDLENDIRTFFPEYTHQLFGDDETAFGYKGLKILLYYIAGSLSTMFRVEYASKVDENFDCVEA
DDVEGKIRQIIPPGFCTNTNDFLSLLEKEVDFKPFGTLLHTYSVLSPTGGENFTFQIYKADMTCRGFREYHERLQTFLMWFIETASF
IDVDDERWHYFLVFEKYNKDGATLFATVGYMTVYNYYVYPDKTRPRVSQMLILTPFQGQGHGAQLLETVHRYYTEFPTVLDITAEDP
SKSYVKLRDFVLVKLCQDLPCFSREKLMQGFNEDMAIEAQQKFKINKQHARRVYEILRLLVTD

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:HAT1 was expressed in E.coli BL21 (DE3) codon plus RIL in 6L Terrific Broth (TB) in the presence of 50 microG/ml of kanamycin. Cell were grown at 37ºC to an OD600 of 1.5 and 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 at ~75000 x g for 20 minutes. 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 250 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 ml/min. Thrombin (Sigma) was added to

combined fractions containing HAT1 and incubated overnight at 4oC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 3.8 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation. The cell pellets were frozen in liquid nitrogen and stored at -80ºC. For the purification, 6L of cells were thawed and resuspended in lysis buffer with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through a Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:23.3 mg/ml
Ligand
MassSpec: Expected mass= 37924.22 Da, measured mass = 38095.6689 Da.
Crystallization: Purified HAT1 was complexed with AcCoA (Sigma) and histone H4 peptide (SGRGKGGKGLGKGGAKRHRK) at 1:10 molar ratio of protein: AcCoA/H4 and crystallized using the sitting drop vapor diffusion method by mixing 1 ul of protein solution with 1 ul of the reservoir solution containing 12% PEG 20K, 0.1M MES pH6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: