Human SF3A1

PDB:1ZKH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:53831993
Entry Clone Source:MGC
SGC Clone Accession:ubh20.704.789; plate SDC009:C5
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:E. coli BL21-(DE3) mgk

Construct

Prelude:
Sequence:

PVSIKVQVPNMQDKTEWKLNGQVLVFTLPLTDQVSVIKVKIHEATGMPAGKQKLQYEGIFIKDSNSLAYYNMANGAVIHLALKERG

Vector:p28a-LIC

Growth

Medium:M9
Antibiotics:
Procedure: Starter cultures from freshly transformed colonies in 20 ml 2xM9 supplemented with zinc and biotin containing ampicillin and kanamycin. This was diluted into a fresh 500 ml 2xM9 media and was grown at 37 degC to a OD600 of around 1.0 and then induced with IPTG to a final concentration of 1 mM, the temperature is lowered to 15degC.

Purification

Procedure
1.2 mL of qiagen Ni NTA bead slurry (50% beads) were added onto the clarified lysate and mixed at 4C for at least 20 min.

Decant and discard the supernatant. Wash the beads twice with 5 mL washing buffer (20 mM Tris-HCl, pH 8.5, 500 mM NaCl, 10 uM ZnSO4, 30 mM immidazole). Elute the protein with 5 ml of elution buffer (20 mM Tris-HCl, pH 8.5, 500 mM NaCl, 10 uM $ZnSO_4$, 500 mM immidazole).

Extraction

Procedure
The cell pellets were thawed and resuspended into lysis buffer (20 mM Tris-HCl, pH 8.5, 500 mM NaCl, 10 uM ZnSO4, 15 mM immidazole). The cell pellets were lysed by sonication and the cell debris were centrifuged.
Concentration:
Ligand
MassSpec:
Crystallization:
NMR Spectroscopy: The NMR sample buffer is 10 mM MOPS, 0.01 % NaN_3, 0.01 mM $ZnSO_4$, 10 mM DTT, 1 mM benzamidine, 1x inhibitor cocktail, 450 mM NaCl, 10% D_2O, pH 6.5.
Data Collection:
Data Processing: