FTHFD

PDB:2BW0

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC027241
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli BL21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMKIAVIGQSLFGQEVYCHLRKEGHEVVGVFTVPDKDGKADPLGLEAEKDGVPVFKYSRWRAKGQA
LPDVVAKYQALGAELNVLPFCSQFIPMEIISAPRHGSIIYHPSLLPRHRGASAINWTLIHGDKKGGFSIFWADDGLDTGDLLLQKEC
EVLPDDTVSTLYNRFLFPEGIKGMVQAVRLIAEGKAPRLPQPEEGATYEGIQKKETAKINWDQPAEAIHNWIRGNDKVPGAWTEACE
QKLTFFNSTLNTSGLVPEGDALPIPGAHRPGVVTKAGLILFGNDDKMLLVKNIQLEDGKMILASNFFK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:20 µL competent BL-21 cells were transformed with 1 µL plasmid. 10min on ice, 45sec at 42°C. Added 100µL SOC then 30min at 37°C. Cells were plated on Kanamycin. Colonies were grown in 50mL of TB + 4% glycerol at 37°C, until OD600 of 0.5 then diluted into 400mL TB + 4% glycerol. Growth at 37°C until OD600 of 1.2 - 1.5. Then at 18 °C for 1h. Induction with 0.5mM IPTG at OD600 of 1.4 - 1.8. Left culture at 18 °C over night.

Purification

Procedure

Extraction

Procedure
Cells were harvested by centrifugation and pellets were resuspended in 50mM HEPES pH 7.5, 500mM NaCl, 10% glycerol, 50µg/mL of Lysosyme was added as well as 1 tablet Complete EDTA-free protease inhibitor tablet per cell pellet. Cells were disrupted by sonication (60%, 1s-1s pulse on-off) for three minutes. DNA precipitation was performed by addition of PEI to a final concentration of 0.15%. The sample was incubated on ice for 30 minutes and centrifuged for one hour at 40000×g. The soluble fraction was filtered through 0.22 µm and subjected to further purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using sitting drop method at 20°C. Drops were prepared using 900 nL of protein (16.5 mg/mL concentration) and 900 nl of the well solution (1.4 M Ammonium Sulfate, 50 mM HEPES pH 7.8). Crystals diffracted to 1.7 Å.

Cryo condition: 2.3 M AmSO4, 20% Glycerol, 0.2M NaCl, 2 mM TCEP, 20mM HEPES pH 7.5, 50mM HEPES pH 7.8
NMR Spectroscopy:
Data Collection:
Data Processing: