

HMGCs1: Human 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1

PDB:2P8U

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:IMAGE:2819708

Entry Clone Source:MGC

SGC Clone Accession:HMGCs1A-c007

Tag:N-terminal His-tag with TEV protease cleavage site (Tag sequence in lower case):

Host:E. coli strain: BL21(DE3)-R3 pRARE2

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfqsmDVGIIVALEIYFPSQYVDQAELEKYDGVDAKYTIGLGQAKMGFCTDREDINSLCMTVVQNLM
ERNLSDYDCIGRLEVGTETIIDKSKSVKTNLMQLFEESGNTDIEGIDTTNACYGGTAAVFNAVNWIESSSWDGRYALVVAGDIA
VYATGNARPTGGVGAVALLIGNAPLIFERGLRGTHMQHAYDFYKPDMLSEYPIVDGKLSIQC YLSALDRCYSVYCKKIHAQWQ
KEGNDKDF TLNDFGFMIFHSPYCKLVQKSLARMLLND FLNDQNRDKNSIYSGLEAFGDVKLEDYF DRDVEKAFMKASSELFSQ
KTKASLLVSNQ NGNMYTSSVYGLASVLAQYSPQQLAGKR IGVSFSGSLAATLYSLKVTQDATPGSAL DKITASLCDLKSRLD
SRTGVAPDVFAENM KLREDTHHLVNYIPQGSIDSLFEGTWYLV RVDEKHRRTYARRP

Vector:pNIC28-Bsa4

Growth

Medium:TB

Antibiotics:

Procedure:10 microL of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50 microG/ml Kanamycin, 34 microG/ml Chloramphenicol) and cultured at 37degC o/n in a shaking incubator (275 rpm). Next day 0.75 ml of o/n culture was used to inoculate 1 litre of TB medium (6x) and grown at 37degC with vigorous shaking (160 rpm) until the culture reaches an OD600 of 1.4. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20degC until further use.

Purification

Procedure

Extraction

Procedure

Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes .

Concentration:

Ligand

Acetyl-CoA **MassSpec:** Corresponds to theoretical mass, as determined by ESI-TOF MS.

Crystallization: Crystals were grown by vapor diffusion in sitting drops at 4degC. Before setting up the experiment acetyl-CoA was added to the protein to a final concentration of 5 mM. A sitting drop consisting of 50 nl protein and 100 nanoliter well solution was equilibrated against well solution containing 25% PEG 3350, 0.2 M ammonium sulfate, 0.1 M BIS-TRIS pH 6.5. The crystal was mounted in a loop directly from the drop and flash-cooled in liquid nitrogen.

NMR Spectroscopy:

Data Collection:

Data Processing: