MDH2

PDB:2DFD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MDH2A-s001 (gi|21735621)
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with TEV protease cleavage site
Host:E.coli strain Rosetta

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMSAQNNA KVAVLGASGGIGQPLSLLLKNSPLVSRLT LYDIAHTPGVAADLSHIETKAAVKGYL
GP EQLPDCLKGCDVVVIPAGVPRKPGMTRDD LFNTNATIVATLTAACAQHCPEAMICVIA NPVNSTIPITAEVFKKHGVYNPNK
IFGVT TLDIVRANTFVAELKGLDPARVNVPVIGG HAGKTIIPLISQCTPKVDFPQDQLTALTG RIQEAGTEVVKAKAGAGSATL
SMAYAGAR FVFSLVDAMNGKEGVVECSFVKSQETECT YFSTPLLLGKKGIEKNLGIGKVSSFEEKM ISDAIPELKASIKKGEDF
VKTLK

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:5 µl of a glycerol stock was inoculated into 5ml of LB medium (supplemented with Kanamycin, 50 µg/ µl) in a 50 ml culture tube and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 1 ml of o/n culture was used to inoculate 1 litre of LB medium and grown at 37°C with vigorous shaking (180 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 cultivated for 16 hrs. Cells were harvested, centrifuged at 6500 rpm for 10 min, and the pellet was stored at -20°C until further use.

Purification

Procedure
Column 1 : 1 ml HisTrap crude (GE/Amersham)
Column 2 : SuperDex 200 16/60 HiLoad (GE/Amersham)

Extraction

Procedure
Thawed cell pellets were dissolved in 30-40 ml of binding buffer (500 mM NaCl, 5%Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole). Cells were lysed using Avestin C-5 microfluidizer, 4 passes . After lysis, the cell lysate was centrifuged at 4°C for 45 minutes at 21,000 (rpm).
Concentration: 10.63 mg/ml using Vivaspin 10K concentrators
Ligand
MassSpec: Corresponds to theoretical mass, as determined by ESI - TOF MS .
Crystallization: Crystals were grown by vapor diffusion at 20°C. Before setting up the experiment, NAD + was added to the protein to a final concentration of 5 mM. A sitting drop consisting of 100 nl protein and 200 nl well solution was equilibrated against well solution containing 30% PEG 1000, 0.1 M MMT pH 6.0. The crystal was transferred to a cryo-protectant comprised of well solution supplemented with 20% glycerol and 2 mM NAD + before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.9Å, X-ray source: Synchrotron SLS -X10, single wavelength.
Data Processing: