UCK1A: Human Uridine-cytidine kinase 1

PDB:2JEO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:
Tag:
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmRPFLIGVSGGTASGKSTVCEKIMELLGQNEVEQRQRKVVILSQDRFYKVLTAEQKAKALKGQYN
FDHPDAFDNDLMHRTLKNIVEGKTVEVPTYDFVTHSRLPETTVVYPADVVLFEGILVFYSQEIRDMFHLRLFVDTDSDVRLSRRVLR
DVRRGRDLEQILTQYTTFVKPAFEEFCLPTKKYADVIIPRGVDNMVAINLIVQHIQDILNGDICKWHRGGS

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:BL21 (DE3) cells from glycerol stocks were grown in 100 mL LB with an addition of 50 µg/mL kanamycin at 37ºC over night. 750 mL of Terrific Broth media supplemented with 50 µg/mL kanamycin was inoculated with 20 mL of the over night culture. The cells were grown in tunair flasks at 37 ºC until OD600 of 1.8-2.0 and were down-tempered to 18 ºC one hour before the induction with 0.5 mM IPTG. Protein expression was allowed to continue over night at 18 ºC. Final OD was 11.6 and the wet cell mass 26.5 g.

Purification

Procedure
Columns:
HiTrap Chelating 1 mL
HiLoadÂ□ 16/60 Superdex 200 Prep Grade

Prior to purification, columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the

HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with IMAC Elution Buffer and loaded on the Gel filtration column connected to an ÄKTAPrime. The chromatogram from gel filtration showed one protein peak. Fractions corresponding to this peak were pooled and the protein was concentrated using VIVASPIN (cut off 10000) to = 10 mg/mL and stored at -80 ºC.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80 ºC. Prior to purification the cell pellet was resuspended in 50 mM HEPES, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP supplemented with one tablet of Complete EDTA-free protease inhibitor and 4 µL/50mL benzonase. Cells were disrupted by sonication according to program #1: 4 s-4 s puls-brake, total run time 6 min. Ultra centrifugation for 30 min at 20500 rpm, 4°C, rotor Sorval SA-800. The supernatants were filtered through 0.2 µm/0.45 µm filters before loading on HisTrap.
Concentration:
Ligand
MassSpec:
Crystallization: The initial protein crystals were obtained using the JCSG screen # 67 (0.8 M Succinic acidpH 7.0) co-crystallized with 5mM CMP. In the optimized condition with 0.8-1.0 M Succinic acid pH 6.7-7.3 , large three dimensional, hexagonal crystals grew after a couple of days using hanging drop vapour diffusion. The structure was solved to 2.5 Å with molecular replacement using the humanuridine-cytidine kinase 2 as template (1XRJ).
NMR Spectroscopy:
Data Collection:
Data Processing: