Cp-GAPDH

PDB:3CPS

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:cgd6_3790
Entry Clone Source:
SGC Clone Accession:cgd6_3790:T4-L339:D4
Tag:mhhhhhhssgrenlyfqg
Host:BL21 (DE3) Ros-Ox

Construct

Prelude:
Sequence:

```
TLGINGFGRIGRLVLRACMERNDITVVAINDPFMDVEYMAYLLKYDSVHGNFNGTVEVSGKDLCINGKVVKVFQAKDPAEIPWGASG
AQIVCESTGVFTTEEKASLHLKGGAKKVIISAPPKDNVPMYVMGVNNTEYDPSKFNVISNASCTTNCLAPLAKIINDKFGIVEGLMT
TVHSLTANQLTVDGPSKGGKDWRAGRCAGNNIIPASTGAAKAVGKVIPALNGKLTGMAIRVPTPDVSVVDLTCKLAKPASIEEIYQA
VKEASNGPMKGIMGYTSDDVVSTDFIGCKYSSIFDKNACIALNDSFVKLISWYDNESGYSNRLVDLAVYVASRGL
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Vector:p28aLIC

Growth

Medium:TB
Antibiotics:
Procedure: The protein was expressed in the host cell in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microgram/mL and 25 microgram/mL, respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microgram/mL and 25 microgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with 50 microgram/mL kanamycin in a 250 mL shaking flask and incubated at 37 °C for 3 hours. Then the culture was transfer into 1.8 L of TB with 50 microgram/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 > 5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and

subsequently onto a 1.0 - 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 - 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 - 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. 5 mM DTT and 1 mM EDTA was added to the eluted CP-GAPDH (cgd6_3790) then it was loaded onto a superdex 200 gel filtration column. The eluted protein(in HEPES, pH 7.5 and 500 mM NaCl) was then concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Stock concentration for CP-GAPDH (cgd6_3790) is 15 mg/mL .

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.
Concentration:28.9 mg/ml
Ligand
MassSpec:expected mass = 36732.6 Da, measured mass = 36733.4 Da
Crystallization:25% glycerolThe protein was crystallized at 20 degC in 20% Peg 3350, 0.2 M Tri-lithium citrate using the Sitting drop method.
NMR Spectroscopy:
Data Collection:
Data Processing: