GAPDH

PDB:1ZNQ

Revision

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Entry Clone Accession:P04406
Entry Clone Source: Dr. William Evans at St Jude Hospital
SGC Clone Accession:
Tag:N-terminal his-tag with integrated thrombin cleavage site: mgsshhhhhhssglvpr*gs
Host: Bl21(DE3), pLysS

Construct

Prelude:
Sequence:

mgshhhhhhssglvprgsHMGKVKVGVNGFGRIGRLVTRAAFNSGKVDIVAINDPFIDLNYMVYMFQYDSTHGKFHGTVKAENGKLV
INGNPITIFQERDPSKIKWGDAGAEYVVESTGVFTTMEKAGAHLQGGAKRVIISAPSADAPMFVMGVNHEKYDNSLKIISNASCTTN
CLAPLAKVIHDNFGIVEGLMTTVHAITATQKTVDGPSGKLWRDGRGALQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTANVSV
VDLTCRLEKPAKYDDIKKVVKQASEGPLKGILGYTEHQVVSSDFNSDTHSSTFDAGAGIALNDHFVKLISWYDNEFGYSNRVVDLMA
HMASKE

Vector:pET15b

Growth

Medium:
Antibiotics:
Procedure: Transformation of the BL21(DE3) pLysS competent cells with the human liver GAPDH construct was done using the heat shock protocol. One colony was picked to start a 50 ml LB culture containing 34ug/ml chloramphenicol (pLysS) and 100ug/ml ampicillin (pET15b). The culture was incubated overnight at 37oC under aerobic conditions with continuous shaking (200 RPM). Forty milliliters of the overnight culture were used to inoculate four liters of LB broth medium containing 34ug/ml chloramphenicol and 100ug/ml ampicillin. The four liters were then equally divided into four flasks (one liter each). The cell cultures were then incubated as above till an OD600 of 0.4 and then induced with 1mM IPTG. After induction, the cells were further grown for three hours. Harvesting the cells was done by centrifuging for 15 minutes at 4,000 RCF (4oC). The cell pellets were then frozen at -20oC till purification step.

Purification

Procedure
The lysate containing N-terminal hexahistidine-tagged GAPDH was applied to a Hi-Trap

chelating column (Amersham Biosciences) charged with nickel sulfate. GAPDH was eluted using a linear gradient of increasing imidazole concentration. Fractions containing GAPDH were then pooled and the His-tag was cleaved by thrombin overnight. The cleaved sample was then subjected to size exclusion chromatography by loading the sample onto a HiLoad 26/60 Superdex 200 gel filtration column (Amersham Biosciences) equilibrated with a buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM EDTA and 1mM DTT. Finally, the GAPDH protein was concentrated to 27 mg/ml with an addition of NAD+ to a final concentration of 0.5mM.

Extraction

Procedure
The frozen cell pellets were thawed and re-suspended in a 100 ml of buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, and 2mM 2-Mercaptoethanol (reducing agent). The cells were then disrupted by microfluidizer. The lysate was then centrifuged at 20,000 RCF for 30 minutes (4oC) and the supernatant was further filtered through cellulose acetate 0.45 um filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using hanging-drop vapour diffusion method. Plate crystals showed after one day in the hanging drop, which contained equal volumes of the protein and reservoir solutions (0.1M sodium formate, and 18% (w/v) PEG 3350 at 18oC).
NMR Spectroscopy:
Data Collection:
Data Processing: