Human Adenylate Kinase 3-Like 1

PDB:1ZD8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:19923437
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPR*GS.
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

```
mgsshhhhhhssglvprgsMGASARLLRAVIMGAPGSGKGTVSSRITTHFELKHLSSGDLLRDNMLRGTEIGVLAKAFIDQGKLIPD
DVMTRLALHELKNLTQYSWLLDGFPRTLPQAEALDRAYQIDTVINLNVPFEVIKQRLTARWIHPASGRVYNIEFNPPKTVGIDDLTG
EPLIQREDDKPETVIKRLKAYEDQTKPVLEYYQKKGVLETFSGTETNKIWPYVYAFLQTKVPQRSQKASVTP
```

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seed cultures by inoculating freshly transformeding E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, all of the seed cultures wasere inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin at 37ºC and grown to an OD600 of 3.0. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in the SGC LEX bubbling system.

Purification

Procedure
The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 3 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4ºC. The Ni-NTA column was washed with 150 mL of the wash buffer (10mM Tris pH7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol.

The protein concentration was estimated based on the extinction coefficient of the protein, 27310 at 280 nm. Five molar equivalents of ADP, 10 mM DTT and 5 mM $MgCl_2$ were added to the purified protein before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 1 mL and the concentration of 50 mg/mL. About 90 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were suspended in 100 mL of the binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification.
Concentration:
Ligand
MassSpec:
Crystallization: Purified His-tagged AK3L1 was crystallized using the sitting drop vapor diffusion method. Crystals grew in one day when the protein (25 mg/mL) was mixed with the reservoir solution at a 1:1 volume ratio. The drop was equilibrated against a reservoir solution containing 30% PEG 4000, 0.1 M sodium citrate, pH 5.6, 0.2 M ammonium acetate
NMR Spectroscopy:
Data Collection:
Data Processing: