

# 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:**

mgsshhhhhssglvprgsMGASARLLRAVIMGAPGSGKGTVSSRITTHFELKHLSSGDLLRDNMLRGTEIGVLAKAFIDQGKLIPD  
DVMTRLALHELKNLTQYSWLLDGFPRTPQAEALDRAYQIDTVINLNPFEVIKQRLTARWIHPASGRVYNIEFNPPKTVGIDDLTG  
EPLIQREDDKPETVIKRLKAYEDQTKPVLEYQKKGVLTFSGTETNKIWPYVYAFQTKVPQRSQKASVTP

**Vector:**p28a-LIC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**We prepared the seed cultures by inoculating freshly transformed E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, all of the seed cultures were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin at 37°C 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 20°C 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 4°C. 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<sub>2</sub> 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 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:**