AMPKB2

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Revision

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Entry Clone Accession:ampkb2.BC053610.MGC.AT79-C12.pCMV-SPORT6
Entry Clone Source:
SGC Clone Accession:ampkb2.070.163; plate SDC043A12
Tag:MGSSHHHHHHSSGLVPR*GS
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPR*GSVKPTQQARPTVIRWSEGGKEVFISGSFNNWSTKIPLIKSHNDFVAILDLPEGEHQYKFFVDGQWVHD
PSEPVVTSQLGTINNLIHVKKSDFEVF

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37ºC to an OD600 of 4.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at room temperature (~22 ºC). The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure
IMAC purification: The clarified supernatant from a 2 L culture was loaded at approximately 1 mL/min by gravity onto 5 mL of Ni-NTA resin (Qiagen 30450). Twelve column volumes of Wash buffer were used at approximately 3 mL/min. Samples were eluted from the Ni-NTA resin by exposure to 8 mL Elution buffer at 1mL/min flow rate. A 15 µL sample of the eluate is saved for SDS-PAGE analysis. 10 µL of each eluate is saved for measurement of protein concentration using Bradford reagent (BioRad 500-0202). (His)6-tag cleavage: Thrombin (Sigma T9681; 1 unit per milligram of protein to 50 U maximum) and CaCl2 (4 mM final concentration) were added to the 8 mL of Ni-NTA eluate in 50 mL conical vials (352096, BD Biosciences) and the conical vial

is stored without shaking, overnight, at 4ºC. Size exclusion chromatography: All gel filtration columns, buffers, and protocols are identical for uncut and thrombin-treated proteins. An XK 16x65 column (part numbers 18-1031-47 and 18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare) is pre-equilibrated with Gel filtration buffer for 1.5 column volumes using an AKTA Express (18-6645-05, GE Healthcare) at a flow rate of 1.5 mL/min. 8 mL of sample is loaded onto the column at 1.5 mL/min, and 2 mL fractions are collected into 96-well plates (VWR 40002-012) using peak fractionation protocols with the following parameters: (Slope; min. peak width 0.833 min; level 0.000 mAU; peak start slope 10.000 AU/min; peak end slope 20.000 AU/min). Peak fractions are analyzed for purity using SDS-PAGE or visual analysis of the chromatogram and pooled. AMPKB2 eluted as multiple peaks; each peak was pooled and concentrated separately and analyzed by mass spectrometry.

Extraction

Procedure
The cell pellet from a 2 L culture was resuspended in 25 mL Lysis buffer and lysed using a Microfluidizer. The lysate was cleared by centrifugation and imidazole added to the clarified supernatant (5 mM final concentration).
Concentration:
Ligand
MassSpec:
Crystallization: Purified AMPKB2 was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (5 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 1.6M NH_4SO_4, 0.2 M NaAc, 0.1 M Hepes, pH 7.5, 5% ethylene glycol in 291 K temperature.
NMR Spectroscopy:
Data Collection:
Data Processing: