AMPKA2

PDB:2H6D

Revision

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Entry Clone Accession:NP_006243
Entry Clone Source:ampka2.006.279; plate SDC062:G8
SGC Clone Accession:
Tag:mgsshhhhhhssglvprGS
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

gsKHDGRVKIGHYVLGDTLGVGTFGKVKIGEHQLTGHKVAVKILNRQKIRSLDVVGKIKREIQNLKLFRHPHIIKLYQVISTPTDFF
MVMEYVSGGELFDYICKHGRVEEMEARRLFQQILSAVDYCHRHMVVHRDLKPENVLLDAHMNAKIADFGLSNMMSDGEFLRTSCGSP
NYAAPEVISGRLYAGPEVDIWSCGVILYALLCGTLPFDDEHVPTLFKKIRGGVFYIPEYLNRSVATLLMHMLQVDPLKRATIKDIRE
HEWFKQDLPSYLFPE

Vector:p28a-thrombin-lic

Growth

Medium: Terrific Broth (TB)
Antibiotics:
Procedure: The host was grown in Terrific Broth (TB) in the presence of kanamycin (50 µg/mL). A stab culture was taken from glycerol stocks and inoculated into 100mL of LB with 50ug/mL kanamycin in a 250mL flask and incubated with shaking at 250rpm overnight at 37 degC. The culture was transfered into 1.8L TB with 50ug/mL kanamycin and 0.6mL of antiforam (Sigma) in 2L bottles and cultured using the LEX system to an OD600 of 4.0-5.0 before induction. The culture was adjusted to 15degC and isopropyl-1-thio-D-galactopyranoside (IPTG) was added to 100ug/mL then incubated overnight at 15degC before harvesting by centrifugation and storage at -80degC.

Purification

Procedure
Imidazole was added to the cleared cell lysate to bring it to a final concentration of 5mM imidazole before loading onto 4 mL bed-volume of Ni-NTA agarose affinity resin (Qiagen #30250). The column was then washed with 50mL of binding buffer and 50mL of Wash

buffer.The Ni-NTA resin was equilibrated in the thrombin cleavage buffer by washing 5-10mL over the resin. After this elution, the stopcock valve was closed. A 10mL volume of thrombin cleavage buffer and 20-40 Units of bovine plasma thrombin was added to the resin. The beads were rocked for 16hours at either 4 degrees or room temperature.The eluate was loaded onto an an XK 16x65 column packed with HighLoad Superdex 200 resin that was pre-equilibrated in gel filtration buffer (AKTAxpress, GE Healthcare). The gel filtration column was run at a flow rate of 1.5mL/min and 2mL fractions collected. Fractions were pooled and concentrated using a centrifugal filter device (Amicon YM10).

Extraction

Procedure
The cell-pellet was thawed, then resuspended in 50mL of binding buffer containing Sigma\'s protease inhibitor cocktail (P2714-1BTL). The thawed cells were homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds. The cells were then lysed by sonication (Virtis408912, Virsonic) on ice with a 10 seconds pulse at half-maximal frequency then 10 seconds rest for a total of 5 minutes sonication time. The resulting lysate was then centrifuged at 63000 xg for 30 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The purified protein was crystallized using a combination of sitting and hanging drop vapor diffusion methods. Protein was mixed with an equal volume of reservoir solution, and the plate incubated at 4 or 18 degC. Crystals were appropriately cryoprotected as indicated below and harvested into liquid nitrogen for data collection.Reservoir: 18.6% PEG 4000, 0.1M AmSO4, 0.1M Tris-HCl pH 8.5, 15%v/v isopropanol, 5mM ATP/MgCl; Cryo: 25% w/v glycerol
NMR Spectroscopy:
Data Collection:
Data Processing: