PKM2

PDB:3U2Z

Revision

Revision Type:created
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Entry Clone Accession:BC007952
Entry Clone Source:MGC: AU36-H1
SGC Clone Accession:HPC002-A01
Tag:N-terminal His6-tag, not removed
Host:BL21-V2R

Construct

Prelude:PANK3: M1-P531
Sequence:

mgsshhhhhhssglvprgsMSKPHSEAGTAFIQTQQLHAAMADTFLEHMCRLDIDSPPITARNTGIICTIGPASRSVETLKEMIKSG
MNVARLNFSHGTHEYHAETIKNVRTATESFASDPILYRPVAVALDTKGPEIRTGLIKGSGTAEVELKKGATLKITLDNAYMEKCDEN
ILWLDYKNICKVVEVGSKIYVDDGLISLQVKQKGADFLVTEVENGGSLGSKKGVNLPGAAVDLPAVSEKDIQDLKFGVEQDVDMVFA
SFIRKASDVHEVRKVLGEKGKNIKIISKIENHEGVRRFDEILEASDGIMVARGDLGIEIPAEKVFLAQKMMIGRCNRAGKPVICATQ
MLESMIKKPRPTRAEGSDVANAVLDGADCIMLSGETAKGDYPLEAVRMQHLIAREAEAAIYHLQLFEELRRLAPITSDPTEATAVGA
VEASFKCCSGAIIVLTKSGRSAHQVARYRPRAPIIAVTRNPQTARQAHLYRGIFPVLCKDPVQEAWAEDVDLRVNFAMNVGKARGFF
KKGDVVIVLTGWRPGSGFTNTMRVVPVP

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The seeds were grown in 80 mL Luria-Bertani broth media supplemented with 50 µg/mL kanamycin at 37 °C overnight. The following morning, all of the seeds were inoculated 1800 mL of Terrific Broth media supplemented with 50 µg/mL kanamycin, 8 g/l glycerol and approximately 500 µl antifoam in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37 °C until OD600nm of 4.0 and were then induced by addition of IPTG to a concentration of 0.5 mM. Protein expression was allowed to continue over night at 18 °C.

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 ºC. The Ni-NTA column was washed with 150 mL of the wash buffer and the protein

was eluted with 15 mL of the elution buffer. The eluate was further purified by size-exclusion chromatography (Superdex 200) equilibrated with 10 mM HEPES pH 7.5, 150mM KCl, 2mM TCEP, 5% glycerol, and 5mM MgCl2. The protein was concentrated using an Amicon Ultra centrifugal filter to the concentration of 50 mg/mL.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were suspended in 100 mL of the binding buffer 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:50 mg/mL
Ligand
6-(3-aminobenzyl)-4-methyl-2-methylsulfinyl- 4,6-dihydro-5H-thieno[2',3':4,5]pyrrolo[2,3-d]pyridazin-5-one**MassSpec:**
Crystallization: Crystallization trials were set up using the vapor diffusion method and the protein drop was equilibrated against a reservoir solution with 1:1 volume ratio. Prior to crystallization, the purified protein was incubated overnight at 4 °C in the presence of the activator (to 5 ~10 mM final concentration). Crystals of activator bound PKM2 were grown at 25% PEG-3350, 0.1M ammonium sulfate, 0.1M Bis-Tris, pH6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: