PKM2

PDB:1ZJH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:33286417
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal histag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:

Construct

Prelude:
Sequence:

```
mgsshhhhhhssglvprgsKPHSEAGTAFIQTQQLHAAMADTFLEHMCRLDIDSPPITARNTGIICTIGPASRSVETLKEMIKSGMN
VARLNFSHGTHEYHAETIKNVRTATESFASDPILYRPVAVALDTKGPEIRTGLIKGSGTAEVELKKGATLKITLDNAYMEKCDENIL
WLDYKNICKVVEVGSKIYVDDGLISLQVKQKGADFLVTEVENGGSLGSKKGVNLPGAAVDLPAVSEKDIQDLKFGVEQDVDMVFASF
IRKASDVHEVRKVLGEKGKNIKIISKIENHEGVRRFDEILEASDGIMVARGDLGIEIPAEKVFLAQKMMIGRCNRAGKPVICATQML
ESMIKKPRPTRAEGSDVANAVLDGADCIMLSGETAKGDYPLEAVRMQHLIAREAEAAIYHLQLFEELRRLAPITSDPTEATAVGAVE
ASFKCCSGAIIVLTKSGRSAHQVARYRPRAPIIAVTRNPQTARQAHLYRGIFPVLCKDPVQEAWAEDVDLRVNFAMNVGKARGFFKK
GDVVIVLTGWRPGSGFTNTMRVVPVP
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Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, all of the seeds were 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 4.0. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in a SGC LEX bubbling system.

Purification

Buffers
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 (10mM Tris pH

7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 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, 29190 at 280 nm. Five molar equivalents of ADP, 5 mM TCEP 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 30 mg/mL. About 55 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Buffers

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:

MassSpec:

Crystallization: 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 (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 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, 29190 at 280 nm. Five molar equivalents of ADP, 5 mM TCEP 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 30 mg/mL. About 55 mg of protein was obtained from 1.8 L of cell culture.

NMR Spectroscopy:

Data Collection:

Data Processing: