

# 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:**

```
mgsshhhhhhssglvprgsKPHSEAGTAFIQTQQQLHAAMADTFLEHMCRLDIDSPPITARNTGIICITGPASRSVETLKEMIKSGMN
VARLNFSHGTHEYHAETIKNVRTATESFASDPILYRPVAVALDTKGPEIRTLIKGSGTAEVELKKGATLKITLDNAYMEKCDENIL
WLDYKNICKVVEVGSKIYVDDGLISLQVKQKGADFLVTEVENGGSLGSKKGVNLPGAADVLPAVSEKDIQDLKFGVEQDVDMVFASF
IRKASDVHEVRKVLGEKGKNIKIISKIENHEGVRRFDEILEASDGIMVARGDLGIEIPAEKVFLAQKMMIGRCNRAGKPVICATQML
ESMIKKPRPTRAEGSDVANAVLDGADCIMLSGETAKGDYPLEAVRMQHЛИAREAAAIYHLQLFEELRR LAPITSDPTEATAVGAVE
ASFKCCSGAIIVLTKSGRSAHQVARYRPRAPIIAVTRNPQTARQAHLYRGIFPVLC KDPVQEAWAEDVDLRVNFAMNVGKARGFFKK
GDVIVLTGWRPGSGFTNTMRVVPVP
```

**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  $\mu$ g/ml of kanamycin at 37 $^{\circ}$ C 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 20 $^{\circ}$ C 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  $^{\circ}$ C. 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<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 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 °C. 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<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 30 mg/mL. About 55 mg of protein was obtained from 1.8 L of cell culture.

#### **NMR Spectroscopy:**

#### **Data Collection:**

#### **Data Processing:**