PIAS3

PDB:4MVT
Entry Clone Accession:BC001154
Entry Clone Source:MGC: AU15-F2
SGC Clone Accession:YTC008-B10
Tag:N-terminal His6-tag, not removed
Host:BL21-V2R
Vector:pET28-MHL
Prelude:PIAS3:E112-H467

Sequence:

mhhhhhhssgrenlyfqgEVDMHPPLPQPVHPDVTMKPLPFYEVYGELIRPTTLASTSSQRFEEAHFTFALTPQQVQQILTSREVLP
GAKCDYTIQVQLRFCLCETSCPQEDYFPPNLFVKVNGKLCPLPGYLPPTKNGAEPKRPSRPINITPLARLSATVPNTIVVNWSSEFG
RNYSLSVYLVRQLTAGTLLQKLRAKGIRNPDHSRALIKEKLTADPDSEVATTSLRVSLMCPLGKMRLTVPCRALTCAHLQSFDAALY
LQMNEKKPTWTCPVCDKKAPYESLIIDGLFMEILSSCSDCDEIQFMEDGSWCPMKPKKEASEVCPPPGYGLDGLQYSPVQGGDPSEN
KKKVEVIDLTIESSSDEEDLPPTKKH

Growth

Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 ug/mL kanamycin at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 16 degree and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm 15min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure: The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatants were loaded onto 5 mL Talon metal-affinity resin column (BD Biosciences). The column was then washed 3 times with 25 mL washing buffer. Bound proteins were eluted using 25 mL elution buffer. Pooled fractions giving a total approximate volume of 25ml were then diluted to 100 ml using Q Buffer A and injected into Q column. Protein was eluted using a linear gradient of 0-100% Q Buffer B over 20 column volumes. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation is tested by SDS-PAGE and >95%.

Extraction

Procedure: 4L cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication using Microfluidizer (Microfluidics M110-EH).

Concentration:26.0 mg/mL

Structure Determination

MassSpec:uncut version native protein expected 41878.1, measured 41878.7.
Crystallization: Initial crystal of PIAS3 was found in JCSG+ condition D06. Crystal of PIAS3 used for structure determination was grown by vapor diffusion at 18°C from a hanging drop consisting of 1.5 ul protein (16.0~26.0 mg/ml, incubated them with chymotrypsin (1:800~1:1300 w/w)) and 1.5 ul well solution containing 0.1 M Tris 8.5, 0.2 M magnesium chloride, 20% PEG8000. The crystal was transferred to a cryo protectant composed of 20% ethylene glycol before flash-cooling in liquid nitrogen.