PIAS2

PDB:4FO9
Entry Clone Accession:BC015190.1
Entry Clone Source:MGC:AU55-E8
SGC Clone Accession:YTC004-C07
Tag:N-terminal His6-tag, not removed
Host:BL21-V2R
Vector:pET28-MHL
Prelude:PIAS2:Q147-R488

Sequence:

mhhhhhhssgrenlyfqgQLKNLPFYDVLDVLIKPTSLVQSSIQRFQEKFFIFALTPQQVREICISRDFLPGGRRDYTVQVQLRLCL
AETSCPQEDNYPNSLCIKVNGKLFPLPGYAPPPKNGIEQKRPGRPLNITSLVRLSSAVPNQISISWASEIGKNYSMSVYLVRQLTSA
MLLQRLKMKGIRNPDHSRALIKEKLTADPDSEIATTSLRVSLMCPLGKMRLTIPCRAVTCTHLQCFDAALYLQMNEKKPTWICPVCD
KKAAYESLILDGLFMEILNDCSDVDEIKFQEDGSWCPMRPKKEAMKVSSQPCTKIESSSVLSKPCSVTVASEASKKKVDVIDLTIES
SSDEEEDPPAKR

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 SP Buffer A and injected into SP column. Protein was eluted using a linear gradient of 0-100% SP 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. The gel shows the preparation contains one major band at ~45 kDa (70%) and a minor band at ~40kDa (30%).

Extraction

Procedure 4L cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication for 10 min (10" on 10" off duty cycle, power at 110W).

Concentration:5.0 mg/mL

Structure Determination

MassSpec:uncut version native protein expected 40575.9, measured 40576.4.minor peak at 36933.55 Da corresponds to 34 a.a. truncation at the C-terminus of the construct.
Crystallization: Initial hit of PIAS2 was from MCSG-II screen condition F4 (1.0 M NaH2PO4/K2HPO4 pH 6.9).The crystal used for data collection was crystallised by vapor diffusion at 18°C from a hanging drop consisting of 1.5ul protein (5.0 mg/ml) and 1.5ul well solution containing 0.7 M KH2PO4/NaH2PO4, pH6.5. The crystal was transferred to a cryo protectant composed of 15% ethylene glycol in well solution before flash-cooling in liquid nitrogen.

The crystals were harvested and checked using mass spectroscopy. 3 major peaks were seen: (40575.1 Da, 36933.9 da, and 35157.8 Da, corresponds to intact, 34 a.a. truncation, and 51 a.a truncation from the C-terminus of the construct).