PNLIPRP1

PDB:2PPL

Revision

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Entry Clone Accession:NP_006220
Entry Clone Source:lipr.BC025784.MGC.AT44E5.pCMVSPORT6
SGC Clone Accession:lipr.018.467:101BA2
Tag: This vector adds 26 amino acids at N terminus (APEHHHHHHDYDIPTTENLYFQGAMD) and 10 amino acids to C terminus (PSLSRSTRGS) of protein of interest.
Host: High-Five insect cells

Construct

Prelude:
Sequence:

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apehhhhhhdydipttenlyfqgamdKEVCYEDLGCFSDTEPWGGTAIRPLKILPWSPEKIGTRFLLYTNENPNNFQILLLSDPSTI
EASNFQMDRKTRFIIHGFIDKGDESWVTDMCKKLFEVEEVNCICVDWKKGSQATYTQAANNVRVVGAQVAQMLDILLTEYSYPPSKV
HLIGHSLGAHVAGEAGSKTPGLSRITGLDPVEASFESTPEEVRLDPSDADFVDVIHTDAAPLIPFLGFGTNQQMGHLDFFPNGGESM
PGCKKNALSQIVDLDGIWAGTRDFVACNHLRSYKYYLESILNPDGFAAYPCTSYKSFESDKCFPCPDQGCPQMGHYADKFAGRTSEE
QQKFFLNTGEASNFARWRYGVSITLSGRTATGQIKVALFGNKGNTHQYSIFRGILKPGSTHSYEFDAKLDVGTIDKVKFLWNNNVIN
PTLPKVGATKITVQKGEEKTVYNFCSEDTVREDTLLTLTPCpslsrstrgs
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Vector:pfhmsp lic n

Growth

Medium: HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802)
Antibiotics:
Procedure:Generation of recombinant virus: Plasmid transfer vector containing target genes were co-transfected into 30-40% confluent monolayer of SF9 cells along with linearized Baculovirus DNA, ProEasyÂ (AB Vector, Cat.#A10S), using Cellfectin transfection reagent (Invitrogen, Cat. No.10362-010). P1 viral stocks were collected after 5 days of incubation at 27 ºC.
Amplification of viral stock: High titers viral stocks were obtained by infecting SF9 cells, at a density of 1 million cells per milliliter of media, with P1 and P2 viral stocks consequently in 6 well plates (Falcon, Cat. No. 353047).
Protein production: HF cells, at density of 2 million cells per milliliter of media, were infected with 1mL of P3 viral stock for each 1L of cell culture. Cell Culture medium was collected after 4 days of incubation on a shaker at 100RPM and 27 ºC.

Purification

Procedure
IMAC purification: The media is spun at 500xg for 3 minutes to pellet the HisLink resin. The supernatant is carefully decanted off the resin, and then 250 mL of IMAC buffer is added to wash the resin. The resin is allowed to settle for 5 minutes, then poured off and washed 3 more times with fresh IMAC buffer. The washed resin is then loaded onto a gravity column, and then washed with a column volume of low imidazole buffer. A 4 µL sample of the low imidazole wash is saved for later analysis by SDS-PAGE. Samples are eluted from the HisLink resin by exposure to 10 mL elution buffer at a 1mL/min flow rate.
HiTrap Q column: IMAC eluate is diluted at least 10X in volume so that the final concentration of NaCl in the buffer is less than 50 mM. The protein is then loaded onto a 5mL HiTrap Q HP column (GE Healthcare, 17-1154-01), washed with three column volumes of Buffer A, and eluted over a linear gradient with Buffer. Peak fractions were pooled and concentrated.
Protein concentration: Purified proteins are concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, Millipore) to a final concentration of 7-10 mg/mL for crystallographic screening and other biophysical studies. The final salt concentration in the protein sample is normalized to 100 mM NaCl by dilution and reconcentration.

Extraction

Procedure
Media is obtained by centrifugation; the media is brought to a pH of ~8 by adding 10X lysis buffer (500 mM Tris pH 8.0 and 5 M NaCl) and verifying pH of final 1X solution (50 m Tris pH8 and 0.5M NaCl). This is then mixed with 2-3 mL of HisLink Protein Purification Resin (Promega V8821) per 250 mL treated media. The mixture is incubated with mixing for at least 20 minutes at 4ºC.
Concentration:
Ligand
MassSpec:
Crystallization: Diffraction-quality crystals were obtained from sitting drop equilibration at 18ºC in the following condition: 20% Peg 4000, 0.1M Sodium Cacodylate pH 6.5, 0.2M Calcium Acetate, 7 mg/mL protein in 0.5µL protein+0.5µL reservoir drops. Parantone-N was used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: