LIPS

PDB:2OXE

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Tag:efvehhhhhhhh
Host: High-Five insect cells

Construct

Prelude:
Sequence:

KEVCYGQLGCFSDEKPWAGTLQRPVKLLPWSPEDIDTRFLLYTNENPNNFQLITGTEPDTIEASNFQLDRKTRFIIHGFLDKAEDSW
PSDMCKKMFEVEKVNCICVDWRHGSRAMYTQAVQNIRVVGAETAFLIQALSTQLGYSLEDVHVIGHSLGAHTAAEAGRRLGGRVGRI
TGLDPAGPCFQDEPEEVRLDPSDAVFVDVIHTDSSPIVPSLGFGMSQKVGHLDFFPNGGKEMPGCKKNVLSTITDIDGIWEGIGGFV
SCNHLRSFEYYSSSVLNPDGFLGYPCASYDEFQESKCFPCPAEGCPKMGHYADQFKGKTSAVEQTFFLNTGESGNFTSWRYKVSVTL
SGKEKVNGYIRIALYGSNENSKQYEIFKGSLKPDASHTCAIDVDFNVGKIQKVKFLWNKRGINLSEPKLGASQITVQSGEDGTEYNF
CSSDTVEENVLQSLYPCefvehhhhhhhh

Vector:pAB-bee-LIC

Growth

Medium: HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802)
Antibiotics:
Procedure:Generation of recombinant virus: Plasmid transfer vector, pAB-bee, containing target gene was 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 degC.
Amplification of viral stock: High titer viral stocks were obtained by infecting SF9 cells at a density of 1 million cells per milliliter of media, with P1 and P2 generation viral stocks in 6 well plates (Falcon, Cat. No. 353047).
Protein production: HighFive 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 at 100RPM and 27 degC.

Purification

Procedure
IMAC purification: The lysate was spun at 500xg for 3 minutes to pellet the HisLink resin. The supernatant was carefully decanted off the resin, and then 250 mL of lysis buffer were added to wash the resin. The resin was allowed to settle for 5 minutes, then poured off and washed 3 more times with fresh lysis buffer. The washed resin is then loaded onto a gravity column and 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.
HiTrap Q column: IMAC eluate was diluted at least 10X in volume so that the final concentration of NaCl in the buffer was less than 50 mM. The protein was 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 B. Peak fractions were pooled and concentrated using15 mL Amicon concentrators with 10,000 MWCO (Millipore) to a final concentration of 10-15 mg/mL for crystallographic screening or other biophysical studies.

Extraction

Procedure
Media preparation: Media was obtained by centrifugation; the media was brought to a pH of ~8 by adding 10X lysis buffer and verifying pH of final 1X solution (50 m Tris pH8 and 0.5M NaCl). This was then mixed with 2-3 mL of HisLink Protein Purification Resin (Promega V8821) per 250 mL treated media. The mixture was incubated with mixing for at least 20 minutes at 4 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The diffracting crystals were obtained from hanging drop vaporization, at 18 degC in the following buffer: 1.17M $(NH_4)_2SO_4$, 0.1M Na-Cacodylate pH5.5, 0.04M NaCl, 15 mg/mL protein. 15% glycerol was used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: