DPP7

PDB:3N0T

Revision

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SGC Clone Accession:DPP7:SDC134-D09:C41107
Tag:N-terminal tag: APEHHHHHHDYDIPTTENLYFQGAMD
Host: Sf9 insect cells

Construct

Prelude:
Sequence:

gamdAPGFQERFFQQRLDHFNFERFGNKTFPQRFLVSDRFWVRGEGPIFFYTGNEGDVWAFANNSGFVAELAAERGALLVFAEHRYY
GKSLPFGAQSTQRGHTELLTVEQALADFAELLRALRRDLGAQDAPAIAFGGSYGGMLSAYLRMKYPHLVAGALAASAPVLAVAGLGD
SNQFFRDVTADFEGQSPKCTQGVREAFRQIKDLFLQGAYDTVRWEFGTCQPLSDEKDLTQLFMFARNAFTVLAMMDYPYPTDFLGPL
PANPVKVGCDRLLSEAQRITGLRALAGLVYNASGSEHCYDIYRLYHSCADPTGCGTGPDARAWDYQACTEINLTFASNNVTDMFPDL
PFTDELRQRYCLDTWGVWPRPDWLLTSFWGGDLRAASNIIFSNGNLDPWAGGGIRRNLSASVIAVTIQGGAHHLDLRASHPEDPASV
VEARKLEATIIGEWVKAARREQQPALRGGPRLSL

Vector:pFHMSP-LIC-N

Growth

Medium:
Antibiotics:
Procedure: Plasmid transfer vector pFHMSP-LIC-N containing the gene was transformed into DH10Bac E.coli cells (Invitrogen) to obtain recombinant viral DNA. SF9 cells were transfected with Bacmid DNA using Cellfectin reagent (Invitrogen), and recombinant baculovirus was generated. Viral stock was amplified from P1 to P3.

Sf9 cells grown in HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802) at density of 3 million cells per milliliter of media and with viability not less then 97 % were infected with 7 mL of P3 viral stock for each 1 L of cell culture. Cell culture medium was collected after 4 days of incubation on a shaker at 100 RPM and 27 °C when cells viability dropped to 25-45 %.

Purification

Procedure
IMAC purification: A 1.6 L volume of medium was mixed with 20 mL pre-equilibrated NiNTA

Superflow beads and stirred (Talboys/Troemner) for 1 hour. The resin was transferred to a 100 mL gravity column, washed with 100 mL of Washing Buffer, and the protein was eluted with 10 mL of Elution Buffer. A second round of NiNTA batch absorption has been performed for increased protein yield. Bound protein was eluted from the IMAC columns with Elution Buffer and loaded onto the Gelfiltration (GF) column. The chromatogram from gel filtration showed one major protein peak that consisted of DPP7 confirmed by SDS-PAGE analysis. The protein was then TEV cleaved to remove the poly histidine tag. TEV was added in the ration of 50:1 DPP7:TEV. The reaction was incubated at 4°C for ~2 days. Cleavage was confirmed by SDS-PAGE analysis and the TEV and tag removed by passing the sample through a 1mL HisTrap FF crude column which had been equilibrated with GF buffer.

Extraction

Procedure
The cultured medium was centrifuged at 14,000 xg for 15 minutes, and the pH of the supernatant was adjusted to 7.5 at room temperature by adding 10x Buffer_A. Protease inhibitors were added to final concentrations of 1 mM phenylmethanesulfonyl fluoride (PMSF, Bioshop) and 2 mM benzamidine hydrochloride (Sigma).
Concentration: Purified protein was concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, Millipore) to a final value of 3.5mg/mL. Average yield was about 2 mg/L.
Ligand
MassSpec:
Crystallization: Protein was incubated with inhibitor with 1 to 50 (protein/inhibitor) molar ratio prior to crystallization.
Crystal used for structure determination were grown in: 2M NH4SO4 ,0.2M NaAc 0.1M Hepes 7.5, 5% MPDCryo solution: 25% Glycerol, 2M NH4SO4 ,0.2M NaAc 0.1M Hepes 7.5, 5% MPD
NMR Spectroscopy:
Data Collection:
Data Processing: