GDAP2

PDB:4UML

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Mammalian Gene Collection (IMAGE Consortium Clone ID 3448678)
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MGHHHHHHSSGVDLGTENLYFQSMDPLGAPSQFVDVDTLPSWGDSCQDELNSSDTTAEIFQEDTVRSPFLYNKDVNGKVVLWKGDVA
LLNCTAIVNTSNESLTDKNPVSESIFMLAGPDLKEDLQKLKGCRTGEAKLTKGFNLAARFIIHTVGPKYKSRYRTAAESSLYSCYRN
VLQLAKEQSMSSVGFCVINSAKRGYPLEDATHIALRTVRRFLEIHGETIEKVVFAVSDLEEGTYQKLLPLYFPRSLK Met1-Lys2
28 The N-terminal residues, MGHHHHHHSSGVDLGTENLYFQS, derive from the vector.

Vector:pFB-LIC-Bse

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication on ice. PEI (polyethyleneimine) was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.Lysis Buffer: 50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP, 1:2000 dilution of protease inhibitor cocktail (Sigma) Purification: Column 1: 5 ml of Ni-Sepharose in a 2 cm diameter gravity flow column. Column 1 Buffers: Binding Buffer: 50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP. Wash Buffer 1: As Binding Buffer except 40 mM imidazole and 1M NaCl.Wash Buffer 2: As Binding Buffer except 60 mM imidazole.Elution Buffer: As Binding Buffer except 250 mM imidazole. Column 1 Procedure: The clarified supernatant was passed through the column. The column was

washed with 50 mL of Binding Buffer, 50 mL each of Wash Buffer 1 and Wash Buffer 2, and 25 mL of Elute Buffer was passed through to elute the protein. The elution and final wash fractions were combined. The N-terminal His tag was removed using TEV protease overnight during dialysis into GF Buffer. Column 2: 5 ml of Ni-Sepharose in a 2 cm diameter gravity flow column.Column 2 Procedure: The dialysed protein was passed through the column. The flow-through and an elution fraction containing 10 mM imidazole in the GF Buffer were combined. Column 3: S200 16/60 Gel Filtration (GE Healthcare) Column 3 Buffers: GF Buffer: 20 mM Tris.HCl pH 7.8, 200 mM NaCl, + 0.5 mM TCEP Column 3 Procedure: The protein was concentrated to 5 ml volume and injected onto the column.

Extraction

Buffers
Procedure
Expression cell line: Sf9 (Spodoptera frugiperda) Expression: Bacmid DNA was prepared after transforming the construct DNA into the DH10Bac cell line. The purified Bacmid DNA was used to transfect adherent Sf9 cells and prepare a first generation baculovirus. The baculovirus was amplified for two further cycles to generate a P2 baculovirus. The P2 baculovirus was used to infect 4 L of Sf9 cells at approx. 2.0 x 106 cells/ml, in 4 x 1000 ml volume approx. in 3 L conical flasks in InsectExpress media (Lonza) (7 ml virus per L of culture). Cell harvest: Cells were harvested by centrifugation after ~48 hours and the pellets resuspended in Lysis Buffer and then frozen at -20°C
Concentration: Fractions from gel filtration were pooled and concentrated to 14.5 mg/ml (measured by 280 nm absorbance).
Ligand
MassSpec: Observed: 25500Expected: 25500
Crystallization: Crystals grew from a mixture of 50 nL protein and 100 nL of a well solution containing 20% PEG3350, 10% ethylene glycol and 0.2 M sodium/potassium tartrate, using the vapour diffusion method.Crystals were equilibrated into reservoir solution plus 20% ethylene glycol before freezing in liquid nitrogen
NMR Spectroscopy:
Data Collection: Data was collected at Diamond synchrotron, beamline I03.
Data Processing: