DDB1

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Revision

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Entry Clone Accession:NP_001914.3
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfq*g. The tag was removed for crystallization.
Host: Hi-Five insect cells

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAKNTRLEIYVVTAEGLRPVKEVGMYGKIAVMELFRP
KGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQDRIGRPSETGIIGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFN
IRLEELHVIDVKFLYGCQAPTICFVYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYHNGD
KYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGRLFMLLLEKEEQMDGTVTLKDLRVELLGETSIAECLTYLDNGVVFVGSRLGD
SQLVKLNVDSNEQGSYVVAMETFTNLGPIVDMCVVDLERQGQGQLVTCSGAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDP
NRETYDTLVLSFVGQTRVLMLNGEEVEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVSQEPKALVSEWKEPQAKNISVASCN
SSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVACLDITPLGDSNGLSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSI
LMTTFESSHYLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIYSSNHKLVFSNVNLKEVNY
MCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYESPRKICYQEVSQCFGVLSSRIEVQDTSGGTTALRPSASTQALSSSV
SSSKLFSSSTAPHETSFGEEVEVHNLLIIDQHTFEVLHAHQFLQNEYALSLVSCKLGKDPNTYFIVGTAMVYPEEAEPKQGRIVVFQ
YSDGKLQTVAEKEVKGAVYSMVEFNGKLLASINSTVRLYEWTTEKDVRTECNHYNNIMALYLKTKGDFILVGDLMRSVLLLAYKPME
GNFEEIARDFNPNWMSAVEILDDDNFLGAENAFNLFVCQKDSAATTDEERQHLQEVGLFHLGEFVNVFCHGSLVMQNLGETSTPTQG
SVLFGTVNGMIGLVTSLSESWYNLLLDMQNRLNKVIKSVGKIEHSFWRSFHTERKTEPATGFIDGDLIESFLDISRPKMQEVVANLQ
YDDGSGMKREATADDLIKVVEELTRIH

Vector:pFBOH-LIC

Growth

Medium:
Antibiotics:
Procedure:Transposition: 2 µL of the construct was added and mixed to 30 µl of DH10Bac competent cells in a sterile 96-well microtitre plate on ice. The plate was left on ice for a further 30 minutes. The heat-shock procedure was done by transferring the plate to a 42 °C water bath for 60 seconds and then returning it to ice for a further 2 minutes. 600 µl of SOC medium (pre-warmed to 37°C) was added to the well and the plate incubated at 37°C for 5 hours. The 2 µl culture mixed with pre-warmed 100 µl SOC, and plated out onto LB agar in a 5.5 cm Petri dish contains Gentamicin (7 µg/mL), Kanamycin (50 µg/mL) and Tetracycline (10 µg/mL), Bluo-gal (

200 µg/ml), and IPTG (40 µg/ml). The plates were incubated at 37°C for 48 hours.

Bacmid preparation: One white colony was picked into 3 mL of LB media, with Gentamicin (7 µg/mL), Kanamycin (50 µg/mL) and Tetracycline (10 µg/mL), in a 24-well block (Qiagen, Cat. 19583) and placed in a shaker (250 rpm) for 18 hours at 37°C. Bacmids were purified with Montage(R) kit (Millipore Cat. LSKB09604).

Generation of P1 recombinant Baculovirus: In a Napflow(R) Class II type A/B3 biosafty cabinet, 50 µl HyQ® SFX-insect serum medium (Hyclone, Cat. SH30278.02) was added into 6 µg bacmid and 3ul cellfectin (Invitrogen Cat. 10362-010). Then bacmid and cellfactin in the medium were mixed and incubated at room temperature for 45 minutes. 1 mL SF9 cells (2 x 105 cells/mL) in HyQ® SFX-insect serum medium was added into the mixture in a 24 well plate (Falcon Cat. 353047). After cells sat at the bottom of the plate, remove supernatant, and 280 µl HyQ® SFX-insect serum medium was added to the plate, then the plate was incubated at 27 °C for 5 hours. In the plate, the supernatant of the mixture was replaced with 0.7 mL Graces insect medium (Invitrogen Cat. 11595-030) contained 10% FBS (Invitrogen Cat.12483-020) and 1% antibiotics (100 µg/mL penicillin, 100 µg/mL streptomycin). Then the plate was incubated in 27 °C for 72 hours. The supernatant was collected.

Generation of P2 recombinant Baculovirus: In a 6 well plate (Falcon Cat. 353047), SF9 cells (1 x 106 cells / mL) in 1.5 mL HyQ® SFX-insect serum medium were infected with 80 µl P1 viruses in 27 °C. The culture was incubated in 27 °C for 48 ~ 72 hours. Supernatant was collected after incubation.

Generation of P3 recombinant Baculovirus: In a 500 mL flask, sf9 cells were added into HyQ® SFX-insect serum medium to reach the desity of 2 x 106 cells / mL. 0.2 mL of P2 recombinant Baculovirus was added into the 200ml culture. The flask was shaken in 27 °C, 130 rpm for 48 hours. Supernatant was collected.

Protein production: 5-10 mL P3 recombinant Baculovirus cells were added into 1 L HyQ® SFX-insect serum medium contained High-Five cells (2 x 106 cells / mL) and Gentamicin (10 µg / mL). The culture was put on a shaker with 100 rpm, at 27 °C for 48 hours. Cells were harvested with centrifuge (4000 rpm, 15 minutes). Harvested cells were washed with cold PBS buffer, then flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads (pre equilibrated in binding buffer) by rocking. After 1 hour incubation at 4ºC, the beads were washed with 50 mL of wash buffer. The protein was eluted using 10mL EB.

Column 2: Gel filtration, HiLoad 16/60 Superdex 75 Prep Grade Procedure: The eluent from from the NiNTA column was loaded onto the gel filtration column in GF buffer at 1 mL/min, fraction size 2mL. The fractions containing protein were identified on a SDS-PAGE gel.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the

cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication and samples were centrifuged for 60 min at 70000 g.
Concentration:10 mg/ml.
Ligand
MassSpec:
Crystallization:0.1 M Bis tris, pH 6.5, 0.2 M Lithium sulfate, 25% PEG 3350, 1:6000Protein:Chymotrypsin , VAPOR DIFFUSION, HANGING DROP, temperature 298K.
NMR Spectroscopy:
Data Collection:
Data Processing: