

WDR92

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Revision

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Entry Clone Accession:

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SGC Clone Accession:MGC AT86-D3

Tag:N terminal tag: MGSSHHHHHHSSGLVPRGS

Host:SF9 insect cells

Construct

Prelude:

Sequence:

mgsshhhhhssglvprgsMSAFEKPQIIAHIQKGFNYTVFDCKWVPCSAKFVTMGNFARGTGVIQLYEIQHGDLKLLREIEKAKPI
KCGTFGATSLQQRYLATGDFGGLHIWNLEAPEMPVYSVKGHKEIINAIDGIGGLGIGEGAPEIVTGSRDGTVKVWDPQKDDPVAN
MEPVQGENKRDCTVAFGNAYNQEERVVCAGYDNGDIKFLDLNMLRWETNIKNGVCSLEFDRKDISMNKL VATSLEGKFHVFDNR
TQHPTKGFASVSEKAHKSTVWQVRHLPQNRELF LTAGGAGGLHLWKYEYPIQRSKKDSEGIEMGVAGSVSLLQNVTLSTQPISSLDW
SPDKRGLCVCSSFDQTVRVLIIVTKLNKI

Vector:pFBoc-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 biosafety cabinet, 50 µl HyQ® SFX-insect serum medium (Hyclone, Cat. SH30278.02) was added into 6

µg bacmid and 3µl cellfectin (Invitrogen Cat. 10362-010). Then bacmid and cellfectin in the medium were mixed and incubated at room temperature for 45 minutes. 1 mL SF9 cells (2×10^5 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 (1×10^6 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 density of 2×10^6 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 (2×10^6 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

IMAC: Unclarified lysate was mixed with 2-3 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4 degC. The mixture was then loaded onto an empty column (BioRad) and washed with 100 ml wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280

Gel filtration chromatography: An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 2.5mL/min. The Eluted sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure

Frozen cell pellet were thawed and protease inhibitors cocktail (Roche) and 0.1% NP40 (final concentration) were added. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.

Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.

Ligand

MassSpec:

Crystallization: Diffraction quality crystals were grown using the following protocol: 1.35M Na/K phosphate, pH7.0, vapor diffusion, sitting drop, temperature 297k.

NMR Spectroscopy:

Data Collection:

Data Processing: