DNM3

PDB:3L43

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:SGC:05-C7
NM_015569.2
Entry Clone Source: Origene FB1503_F12
SGC Clone Accession:HPC09I-C03
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:DYN3:M6-S306
Tag was not removed
Sequence:

mhhhhhhssgrenlyfqgMEELIPLVNRLQDAFSALGQSCLLELPQIAVVGGQSAGKSSVLENFVGRDFLPRGSGIVTRRPLVLQLI
TSKAEYAEFLHCKGKKFTDFDEVRLEIEAETDRVTGMNKGISSIPINLRVYSPHVLNLTLIDLPGITKVPVGDQPPDIEYQIREMIM
QFITRENCLILAVTPANTDLANSDALKLAKEVDPQGLRTIGVITKLDLMDEGTDARDVLENKLLPLRRGYVGVVNRSQKDIDGKKDI
KAAMLAERKFFLSHPAYRHIADRMGTPHLQKVLNQQLTNHIRDTLPNFRNKLQGQLLS

Vector:pET28-mhl (GI:134105571)

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 °reeC and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °reeC.

Purification

Procedure
The lysate was centrigued at 16,000 rpm for 1 hour and the supernatants were mixed with 8 mL 50% flurry of Ni-NTA beads and incubated at 4°reeC on rotary shaker for one hour. The mixture was then centrifuged at 3000 rpm for 3 min and the supernant discarded. The beads were then

washed with washing buffer containing 20 mM Imidazole, and eluted with the elution buffer. The flow-trough was collected and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (mw cut-off 10k). The purity of the preparation is tested by SDS-PAGE to be greater than 90%.

During purification, the tag was not removed.

Extraction

Procedure
Frozen cells from 6L TB culture were thawed and resuspended in 1000 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with 1.6 mL protease inhibitor cocktail (SIGMA Catalog # P8849), and 10 µL benzonase (Sigma Catalog # E1014, 250U/µL), 1mM PMSF/Benzamidine, and lysed using sonication at 120W for 6\'.
Concentration:25.9 mg/mL
Ligand
MassSpec: Native expected 35792.28, measured 35815.59 (delta +23.3, sample overloaded on mass)
Crystallization: Crystallization was setup using in situ proteolysis method in sitting drops with Red Wings and SGC-I screens initially. Crystals were found in RW-A3#2 and A10#2 (subtilisin), SGC-A9#1 (no protease) Diffracting crystals were found from initial screen drops.Crystal used for structure determination was grown in 25% PEG3350, 0.2M NaCl, 0.1 M HEPES, pH 7.5, with 1:100 Subtilisin A (w/w) in sitting drop setup (0.5uL+0.5uL).
Crystals grow to a mountable size with a few days.
0.9V well solution plus 0.1V 80% glycerol was used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: