MALT1-DD

PDB:2G7R

Revision

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Entry Clone Accession:malt1.BC030143.MGC.AU79-A6.pOTB7
Entry Clone Source:
SGC Clone Accession:malt1.029.126; plate SDC34H07
Tag:MGSSHHHHHHSSGLVPRGS
Host:E. coli. BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsTLNRLREPLLRRLSELLDQAPEGRGWRRLAELAGSRGRLRLSCLDLEQCSLKVLEPEGSPSLCLLKLM
GEKGCTVTELSDFLQAMEHTEVLQLLSPPG

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: Media bottles (2 L) containing TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ ml kanamycin and 600 ul antifoam 204 (Sigma A-8311) were inoculated with 50-100 ml of the overnight LB culture each. With sterilized cap/sparger (Fisher 11-138B) assemblies, bottles were placed into a circulating water bath set at 37 degC. Temperature was reduced to 15oC one hour prior to induction at OD(600) between 4 and 8 with 100 uM isopropyl-thio-B-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 degC.

Purification

Procedure
Three milliliters of TALON metal-affinity resin (BD Bioscience) was mixed for 2 hours at 4 degC with 40 mL lysate, centrifuged for 3 minutes (SX4750 rotor, Allegra X-12R, Beckman Coulter), and decanted. Beads were transferred into a 25 mL Econo-Column (Bio-Rad 732-1010) and washed with 5 x 12 mL Wash buffer. Samples are eluted with 2-3 column volumes of Elution buffer. EDTA was added up to 1 mM to each protein sample followed by 2 mM DTT. Samples were gel-filtered (XK 16x65 packed with HighLoad Superdex 200 resin, GE Healthcare) using an

AKTAxpress (18-6645-05, GE Healthcare) at a flow rate of 1 mL/min Gel-filtration buffer, and 2 mL fractions were collected in 96-well plates. Fractions containing protein were pooled and centrifuged through concentrators with 5,000 kDa cut-off (Amicon Ultra-15, UFC900524, Millipore) for 45 minutes at 3750 rpm.

Extraction

Procedure
Frozen cell pellets from 2L cultures contained in bags (Beckman 369256) were thawed by soaking in warm water, resuspended in 25-40 mL Lysis buffer, and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds. Cell lysis was accomplished with a Microfluidizer Processor M-110EH (Microfluidics) at 18,000-20,000 psi with ice cooling. Aliquots of PMSF were added (60-80 µL of 100 mM stock) prior to centrifugation at 10°C for 30 minutes (JA25.50 rotor at 23,000 rpm). Supernatants were decanted into sterile 50-ml polypropylene conical tubes.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 298 K using the hanging drop method by mixing equal volumes of 1.0 M K/Na tartrate, 0.1 M MES pH 6.0 and 10 mg/mL protein. Crystals did not require cryoprotection before freezing in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: