MALT1

PDB:3BFO

Revision

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Entry Clone Accession:NP_006776
Entry Clone Source:malt1.BC030143.MGC.AU79-A6.pOTB7
SGC Clone Accession:malt1.226.314.128B09 (SDC128B09)
Tag:N-terminal: MGSSHHHHHHSSGLVPR*GS (removed)
Host:E.coli BL21(DE3)

Construct

Prelude:
Sequence:

```
mgsshhhhhhssglvpr*GSKLQICVEPTSQKLMPGSTLVLQCVAVGSPIPHYQWFKNELPLTHETKKLYMVPYVDLEHQGTYWCHV
YNDRDSQDSKKVEIIIDELNNL
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Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37ºC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure:

IMAC: The cleared lysate from a 2 L culture was loaded onto 3 ml TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed with 40 ml Wash buffer, and the protein was eluted with 10 ml Elution buffer.
Tag removal: 1 Unit of thrombin (Sigma T9681) per milligram of protein was added to the 10 mL sample, stored overnight without shaking at 4ºC.
Gel-filtration: It was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer and concentrated to 50 mg/ml by ultrafiltration using Amicon Ultra centrifugal filter with 10 kD cutoff and stored at -80 ºC.
Protein yield was 12 mg per liter of bacterial culture.

Extraction

Procedure: The cell pellet was defrosted and cells were lysed by sonication, 10 s on, 10 s off at 40% amplitude for 10 min. The lysate was cleared by centrifugation for 45 minutes at 15,500 RPM, 4°C.
Concentration:50 mg/ml
Ligand:
MassSpec: Mass-spectroscopy by LCMS shows that the product was pure and of correct molecular weight.
Crystallization: Purified protein was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (50 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 30% PEG 550-MME, 0.2 M Ammonium sulfate, and 0.1 M Sodium cacodylate at pH 6.5 in 293 Kelvin.
NMR Spectroscopy:
Data Collection:
Data Processing: