LTB4DH

PDB:1ZSV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:LTB4DHA -s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N terminal histag with TEV cleavage site: mhhhhhhssgvdlgtenlyfq*s
Host:

Construct

Prelude:
Sequence:

SMTKTWTLKKHFVGYPTNSDFELKTSELPPLKNGEVLLEALFLTVDPYMRVAAKRLKEGDTMMGQQVAKVVESKNVALPKGTIVLAS
PGWTTHSISDGKDLEKLLTEWPDTIPLSLALGTVGMPGLTAYFGLLEICGVKGGETVMVNAAAGAVGSVVGQIAKLKGCKVVGAVGS
DEKVAYLQKLGFDVVFNYKTVESLEETLKKASPDGYDCYFDNVGGEFSNTVIGQMKKFGRIAICGAISTYNRTGPLPPGPPPEIVIY
QELRMEAFVVYRWQGDARQKALKDLLKWVLEGKIQYKEYIIEGFENMPAAFMGMLKGDNLGKTIVKA

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: The LTB4DH construct was expressed in E. coli in 1 L LB medium in the presence of 100 µg/mL of ampicillin at 37°C. Cells were induced at 1 mM IPTG as soon as the OD reached 0.6, and temperature was shifted to 18°C, and the culture was grown for 12 hrs. Cells were collected by centrifugation, and pellets were stored frozen (-20°C) until further use.

Purification

Procedure
Column 1: HiTrap His

Buffers (adjusted to pH 8.0): Lysis buffer: 10mM Imidazole, 300mM NaCl, 50mM NaH2PO4; Wash buffer: 20mM Imidazole, 300mM NaCl, 50mM NaH2PO4 ; Elution Buffer: 250mM Imidazole, 300mM NaCl.

Procedure: Sample was loaded, washed with wash buffer and eluted in elution buffer. The protein was concentrated and a buffer exchange into 10 mM Hepes, pH 7.4, 500 mM NaCl, 5% glycerol

was performed using an Amicon Ultra device.

Extraction

Procedure
Pellets were resuspended in 20 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, nucleic acids were removed by 0.15% PEI precipitation, and the solution was centrifuged to obtain a clear supernatant (15 min, 20,000 x g). Supernatants were processed in a 2 step chromatographic procedure using the Akta xpress (GE Healthcare) purification system.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1: HiTrap His

Buffers (adjusted to pH 8.0): Lysis buffer: 10mM Imidazole, 300mM NaCl, 50mM NaH_2PO_4; Wash buffer: 20mM Imidazole, 300mM NaCl, 50mM NaH_2PO_4 ; Elution Buffer: 250mM Imidazole, 300mM NaCl.

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NMR Spectroscopy:
Data Collection:
Data Processing: