FABP1

PDB:2F73

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC032801
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli Bl21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMSFSGKYQLQSQENFEAFMKAIGLPEELIQKGKDIKGVSEIVQNGKHFKFTITAGSKVIQNEFTV
GEECELETMTGEKVKTVVQLEGDNKLVTTFKNIKSVTELNGDIITNTMTLGDIVFKRISKRI

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:30 µL competent Bl21(DE3) cells (Novagen) were transformed with 1 µL plasmid. Held 30min on ice, heat-shocked in 42 degree waterbath for 45sec at 42°C. Held on ice for 2 min. Added 100µL SOC and incubated in shaker for 1 h. Cells were plated on LA plates with 50 mg/l Kanamycin and 0.2% glucose. Glycerol stocks were made by adding 5 colonies to 1ml phosphate buffered TB with 50 mg/l Kanamycin. 150 ul culture was taken out at mid-logphase (3 hours growth at 37 degrees) and mixed with sterile glycerol to a final concentration of 20% and then frozen at -80. The glycerol stock was used to inoculate 4 ml of SOC + Kan 50 mg/l in a 50 ml Falcon tube. The inoculation culture was shaken at 37 degrees for 3 hours when it was added to a TunAir flask (Shelton Scientific) with 750 ml of phosphate buffered TB with 50 mg/l Kanamycin. The culture was incubated at 37°C, until OD600 reached of approximately 1.4. The temperature was lowered to 18°C and the culture was induced with 0.5 mM IPTG for 15 hours.

Purification

Procedure
Buffers: 50 mM HEPES, pH 7.5, 10 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP

(IMAC Bind/Wash1 Buffer); 50 mM HEPES, pH 7.5, 50 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Wash2 Buffer); 50 mM HEPES, pH 7.5, 400 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Elution Buffer).

Columns: 1 ml Hi-Trap Chelating (Ni-charged). (GE Healthcare). Superdex 75 HiLoad 16/60 (GE Healthcare).

Procedure: The sample was purified automatically on an ÄKTA-Xpress (GE Healthcare). Briefly, sample was loaded on the IMAC column, eluted in a storage loop and then loaded on the gel filtration column. Elution fractions were pooled based on SDS-PAGE analysis. Protein was estimated by SDS-PAGE analysis to be more than 95% pure. Fresh TCEP was added to the pooled samples so that the concentration of TCEP was 2 mM. Concentration was performed by use of Amicon Ultra 15 (Millipore) with 10 000 MW CO. Centrifugation was performed at 15 deg in swing-out buckets, in 5 minutes intervals and maximum 15 minutes, at 3000 g. Yield of purified protein per liter of culture was 4.9 mg.

Extraction

Procedure
Cells were harvested by centrifugation (OD600 13.4; WCW 18 g) and pellets were resuspended in 30 ml of lysis buffer (50mM HEPES pH 7.5, 500mM NaCl, 10% glycerol, 10 mM Imidazole, 0.5 mm TCEP and 1 tablet Complete EDTA-free protease inhibitor (Roche Biosciences)). A knife edge of lysozyme (Sigma) was added before freezing at -20.
After thawing, 4 µL of a 250 U/µl benzonase (Novagen) stock solution was added and lysis buffer was added to a total volume of 70 ml. Cells were then disrupted by high pressure homogenization with a high-pressure homogenizer (Stansted) (4 passes) prior to centrifugation for 30 min at 49000 g in a Sorvall SA-800 rotor. The soluble fraction was decanted and filtered through 0.22 µm.
Concentration:
Ligand
MassSpec:
Crystallization: Buffers: 50 mM HEPES, pH 7.5, 10 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Bind/Wash1 Buffer); 50 mM HEPES, pH 7.5, 50 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Wash2 Buffer); 50 mM HEPES, pH 7.5, 400 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Elution Buffer).

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NMR Spectroscopy:
Data Collection:
Data Processing: