UXS1

PDB:2B69

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:UXS1A-s001
Entry Clone Source:synthetic, codon optimized for E.coli expression
SGC Clone Accession:
Tag:
Host:B834(DE3) (Methionine auxotroph)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghmEKDRKRILITGGAGFVGSHLTDKLMMDGHEVTVVDNFFTGRKRNVEHWIGHENFELINHDVVEP
LYIEVDQIYHLASPASPPNYMYNPIKTLKTNTIGTLNMLGLAKRVGARLLLASTSEVYGDPEVHPQSEDYWGHVNPIGPRACYDEGK
RVAETMCYAYMKQEGVEVRVARIFNTFGPRMHMNDGRVVSNFILQALQGEPLTVYGSGSQTRAFQYVSDLVNGLVALMNSNVSSPVN
LGNPEEHTILEFAQLIKNLVGSGSEIQFSEAQDDPQKRKPDIKKAKLMLGWEPVVPLEEGLNKAIHYFRKELEYQANNQgs

Vector:p11

Growth

Medium:
Antibiotics:
Procedure:B834(DE3) cells were transformed with the UXS1A plasmid and grown on Lb Amp plates. A single colony was used to start a 5mL overnight culture in LB/Amp, and 1mL of the overnight culture was used to inoculate 50mL of LB medium, grown at 37°C to an OD of 1.0. The cells were harvested by centrifugation, washed four times in MD medium (SelenoMet Medium Base, SelenoMet Nutrient, selenomethionine to final concentration of 40 mg/L, Molecular Dimensions) and finally the cells were inoculated in 1L of MD medium, and grown at 37°C to an OD 0.6. The culture was induced by the addition of IPTG to a final concentration of 1mM, cultured overnight at 25°C, and collected by centrifugation.

Purification

Procedure
Column 1 : Ni-NTA resin

Buffers (adjusted to pH 8.0): Lysis buffer: 5 mM Imidazole, 500mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol; Wash buffer: 30 mM Imidazole, 500 mM NaCl, 50mM HEPES, pH 7.5, 5%

glycerol; Elution Buffer: 250 mM Imidazole, 500 mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol.

Column 2: Superdex S200

Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 1mM TCEP

Procedure: Sample was loaded, washed with wash buffer and eluted in elution buffer. The protein was concentrated using an Amicon Ultra device.

Extraction

Procedure
Pellets were resuspended in 20 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, and the solution was centrifuged to obtain a clear supernatant (30 min, 20.000 x g). Supernatants were processed in a 2 step chromatographic procedure.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Ni-NTA resin

Buffers (adjusted to pH 8.0): Lysis buffer: 5 mM Imidazole, 500mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol; Wash buffer: 30 mM Imidazole, 500 mM NaCl, 50mM HEPES, pH 7.5, 5% glycerol; Elution Buffer: 250 mM Imidazole, 500 mM NaCl, 50 mM HEPES, pH 7.5, 5% glycerol.

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NMR Spectroscopy:
Data Collection:
Data Processing: