CRYZ

PDB:1YB5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:13236495
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*gh(m)
Host: Rosetta-2 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghmMATGQKLMRAVRVFEFGGPEVLKLRSDIAVPIPKDHQVLIKVHACGVNPVETYIRSGTYSRKPL
LPYTPGSDVAGVIEAVGDNASAFKKGDRVFTSSTISGGYAEYALAADHTVYKLPEKLDFKQGAAIGIPYFTAYRALIHSACVKAGES
VLVHGASGGVGLAACQIARAYGLKILGTAGTEEGQKIVLQNGAHEVFNHREVNYIDKIKKYVGEKGIDIIIEMLANVNLSKDLSLLS
HGGRVIVVGSRGTIEINPRDTMAKESSIIGVTLFSSTKEEFQQYAAALQAGMEIGWLKPVIGSQYPLEKVAEAHENIIHGSGATGKM
ILLL

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: The CRYZ construct was expressed in the Rosetta strain of BL21 in 50 mL Terrific Broth (TB at 5% glucose) in the presence of 100 µg/mL of ampicillin and 34 µg/ml chloramphenicol at 37ºC overnight. Cells were collected by centrifugation and resuspended into 10 ml prewarmed TB medium (containing 0.05% glucose, 0.2% lactose, 0.06% glycerol, and antibiotics). 25% of this culture was used to inoculate 2x 500 ml of the same medium, and then grown to an OD of 2 at 37ºC. Temperature was shifted to room temperature and the cultures were grown for 20 hrs and reached an OD>15. Cells were collected by centrifugation, and pellets were stored frozen (-20) until further use.

Purification

Procedure
Affinity column: His bind resin. Buffers adjusted to pH 8.0. Lysis buffer: 10 mM imidazole, 300 mM NaCl, 50 mM NaH_2PO_4. 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 collected peak was injected into size-exclusion chromatography system, and the main peak eluting at about 70K was selected for concentration using an Amicon Ultra device.

Gel filtration: Superdex S200. Buffer: 10 mM Hepes, pH 7.4, 500 mM NaCl, 5% glycerol

Extraction

Procedure
Pellets were resuspended in 20 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, and centrifuged to obtained 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: Crystals were grown at 20 ºC in 150 nL sitting drops by mixing 100 nL of protein solution (including about 5 mM NADPH) and 50 nL of crystallisation solution consisting of 14% PEG10K, 160 mM Ca-acetate, 0.8 M cacodylate, pH 6.5, 20% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: