

SPR

PDB:1Z6Z

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:gi:4507185

Entry Clone Source:Synthetic

SGC Clone Accession:

Tag:mgsshhhhhssgrenlyfq*gh (N terminus); gs (C terminus); TEV protease cleavable at *

Host:BL21 (DE3)

Construct

Prelude:

Sequence:

EGGLGRAVCLLTGASRGFGRTLAPLLASLLSPGSVLVLSARNDALRQLEAELGAERSGLRVVRVPADLGAEAGLQQLLGALRELPR
PKGLQRLLLINNAGSLGDVSKGFVDLSDSTQVNNYWALNLTSMCLCTSSVLKAFDPSPGLNRTVVNISSLCALQPFGWALYCAGKA
ARDMLFQVLALEEPNVRVLNYAPGPLDMDQQLARETSVDPDMRKGLQELKAKGKLVDCVSAQKLLSLLEKD

Vector:p11

Growth

Medium:

Antibiotics:

Procedure:Starter culture in 3 ml of LB + 100 µg/mL. Terrific Broth + 100 µg/mL, with in-house autoinduction componenets. Cultures grown for 7 hours at 37 °C and the temperature reduced to 15 °C overnight.

Purification

Procedure

Affinity column: 1 mL His-Trap (GE/Amersham). Buffers: 50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole; 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM imidazole; 50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 250 mM imidazole.

Procedure: AKTA Xpress Affinity/Gel Filtration

Gel filtration: SuperDex 200 16/60 HiLoad (GE/Amersham). Buffers: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol. Procedure: AKTA Xpress Affinity/Gel Filtration

Extraction

Procedure

50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole, PMSF to 1 mM added. Cell pellet was resuspended in the above buffer then frozen, the thawed resuspension was lysed using Avestin C-5 microfluidizer, 4 passes. PEI pH 8 added to a final concentration of 0.05% and spun in a JA-17 rotor at 17,000 rpm.

Concentration:**Ligand****MassSpec:**

Crystallization: Affinity column: 1 mL His-Trap (GE/Amersham). Buffers: 50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole; 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM imidazole; 50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 250 mM imidazole. Procedure: AKTA Xpress Affinity/Gel Filtration

Gel filtration: SuperDex 200 16/60 HiLoad (GE/Amersham). Buffers: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol. Procedure: AKTA Xpress Affinity/Gel Filtration

NMR Spectroscopy:**Data Collection:****Data Processing:**