retSDR3

PDB:1YDE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:7705907
Entry Clone Source: Synthetic
SGC Clone Accession:
Tag:N-term: gsshhhhhhssgrenlyfqghm. C-term: gs
Host: Rosetta-2 (DE3)

Construct

Prelude:
Sequence:

ATGTRYAGKVVVVTGGGRGIGAGIVRAFVNSGARVVICDKDESGGRALEQELPGAVFILCDVTQEDDVKTLVSETIRRFGRLDCVVN
NAGHHPPPQRPEETSAQGFRQLLELNLLGTYTLTKLALPYLRKSQGNVINISSLVGAIGQAQAVPYVATKGAVTAMTKALALDESPY
GVRVNCISPGNIWTPLWEELAALMPDPRASIREGMLAQPLGRMGQPAEVGAAAVFLASEANFCTGIELLVTGGAELGYGCKASRSTP
VDAPDIPS

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: Medium: TB, 34 µg/mL kanamycin and 100 µg/mL ampicillin. 1 liter TB in 2.5 L baffled flasks was inoculated with an overnight culture. The culture was grown at 37 °C to OD of 0.6 and then transfered to 15 °C. 1 mM IPTG was then added, and incubation continued over a period of 20 hours. The cells were then collected by centrifugation and frozen at -80°C

Purification

Procedure
Buffers for DE-52 and Ni-NTA columns: Binding buffer (BB): 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole. Wash buffer (WB): 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% glycerol, 30 mM imidazole. Elution buffer (EB): 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 250 mM imidazole. Procedure: Gravity feed chromatography. Sample applied to a 10 ml DE-52 column and washed through with 20 mL BB. The flow through was applied to a 2 mL Ni-NTA column, the Ni-NTA column was washed with 200 mL of WB and eluted with EB

in 2 mL aliquots. Eluate was monitored for protein using Coomassie Blue Plus protein assay reagent. Procedure: As above.

Gel Filtration: S75 16/60 prep grade.

GF buffer: 10 mM HEPES, pH 7.5 500 mM NaCl, 5% glycerol, 0.5 mM TCEP. Procedure: The eluted fraction was loaded and fractionated on the gel filtration column in GF buffer at 1 mL/min.

Extraction

Procedure
Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole, PMSF to 1 mM added.
Concentration:
Ligand
MassSpec:
Crystallization: Magnesium acetate, MPD, 0.1M cacodylate buffer, pH 6.5, vapour diffusion, hanging drop, temperature 293K
NMR Spectroscopy:
Data Collection:
Data Processing: