RETSDR2

PDB:1YB1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:7705905
Entry Clone Source: Synthetic
SGC Clone Accession:
Tag:N-term histag with integrated TEV cleavage site: gsshhhhhhssgrenlyfq*gh(m). C-term: gs
Host: Rosetta2(DE3)

Construct

Prelude:
Sequence:

```
PKRRKSVTGEIVLITGAGHGIGRLTAYEFAKLKSKLVLWDINKHGLEETAAKCKGLGAKVHTFVVDCSNREDIYSSAKKVKAEIGDV
SILVNNAGVVYTSDLFATQDPQIEKTFEVNVLAHFWTTKAFLPAMTKNNHGHIVTVASAAGHVSVPFLLAYCSSKFAAVGFHKTLTD
ELAALQITGVKTTCLCPNFVNTGFIKNPSTSLGPTLEPEEVVNRLMHGILTEQKMIFIPSSIAFLTTLERILPE
```

Vector:p11

Growth

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

Purification

Procedure
Ni-affinity: HisTrap, 1 mL (GE/Amersham). Loading buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP. Procedure: The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Loading buffer, 10 volumes of wash buffer, then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Gel Filtration: SuperDex 200.

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 1.2 ml/min.

Extraction

Procedure
Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, and 0.5mM PMSF. Frozen cell pellets were thawed in a total volume of 30 mL lysis buffer, the cells were disrupted by homogenisation. (15kpsi). Nucleic acids and cell debris were removed by adding 0.15% PEI from a 5% (w/v) stock, stirring for 15 minutes, then centrifugation for 20 minutes at 25,000xg. The supernatant was then further clarified by filtration (first 1.2 µm and then 0.2 µm).
Concentration:
Ligand
MassSpec:
Crystallization:0.08M Sodium Citrate pH5, 2.52M Ammonium Sulphate, Vapour Diffusion, Sitting Drop, Temperature 293K.
NMR Spectroscopy:
Data Collection:
Data Processing: