PECR

PDB:1YXM

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:19923817
Entry Clone Source: Synthetic
SGC Clone Accession:
Tag:mgsshhhhhhssgrenlyfq*gh(N terminus), gs (C terminus), TEV protease cleavable at *
Host:BL21(DE3)

Construct

Prelude:
Sequence:

MASWAKGRSYLAPGLLQGQVAIVTGGATGIGKAIVKELLELGSNVVIASRKLERLKSAADELQANLPPTKQARVIPIQCNIRNEEEV
NNLVKSTLDTFGKINFLVNNGGGQFLSPAEHISSKGWHAVLETNLTGTFYMCKAVYSSWMKEHGGSIVNIIVPTKAGFPLAVHSGAA
RAGVYNLTKSLALEWACSGIRINCVAPGVIYSQTAVENYGSWGQSFFEGSFQKIPAKRIGVPEEVSSVVCFLLSPAASFITGQSVDV
DGGRSLYTHSYEVPDHDNWPKGAGDLSVVKKMKETFKEKAKL

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: Starter culture in 3 ml of LB + 100 µg/mL ampicillin. Large scale: Terrific Broth + 100 µg/mL ampicillin. The cultures were then grown until OD 0.6-0.8 at 37oC with shaking, and induced with 1.5 ml 100 mM IPTG, then grown at 17oC for overnight.

Purification

Procedure
DE-52/Ni-NTA buffers: Bindingbuffer (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: TEV clean up. The TEV cleaved protein was applied to a 1 mL

Ni-NTA column and washed through with BB. The flow-through being collected. The eluate from the column was monitored and when all the unbound protein had flowed through. The column was eluted with EB into a fresh container.

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, in 4 passes. Lysate spun in a JA-17 rotor at 17,000 rpm.
Concentration:
Ligand
MassSpec:
Crystallization:JCSG+ screen conditions at 20 ºC: E1 and E2, E2 offering superior diffraction. Protein concentration 10 mg/mL with NADPH to 2 mM.Well solution: 0.1 M sodium cacodylate pH 6.5, 2 M ammonium sulphate, 0.2 M sodium chloride.
NMR Spectroscopy:
Data Collection:
Data Processing: