PRKCL2

PDB:4CRS

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal His6-Z-TEV
Host: Insect cells (Sf9)

Construct

Prelude:
Sequence:

MGHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLRDDPSQSANLLAEAKKLNDAQPKGTENLYFQSMSQQRFQFNL
QDFRCCAVLGRGHFGKVLLAEYKNTNEMFAIKALKKGDIVARDEVDSLMCEKRIFETVNSVRHPFLVNLFACFQTKEHVCFVMEYAA
GGDLMMHIHTDVFSEPRAVFYAACVVLGLQYLHEHKIVYRDLKLDNLLLDTEGFVKIADFGLCKEGMGYGDRTSTFCGTPEFLAPEV
LTETSYTRAVDWWGLGVLIYEMLVGESPFPGDDEEEVFDSIVNDEVRYPRFLSTEAISIMRRLLRRNPERRLGASEKDAEDVKKHPF
FRLIDWSALMDKKVKPPFIPTIRGREDVSNFDDEFTSEAPILTPPREPRILSEEEQEMFRDFDYIADWC residues marked i
n bold are cleaved by TEV protease

Vector:pFB-6HZB

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
A 15 g cell pellet was thawed, resuspended in 100 mL lysis buffer (50 mM HEPES/NaOH pH 7.4, 500 mM NaCl, 20 mM imidazole, 0.5 mM TCEP) and sonicated (2 minutes, amplitude 35%, on ice). The cell lysate was cleared by centrifugation (45 minutes, 100,000x g, 4°C). The supernatant was combined with 5 mL Ni Sepharose and loaded onto a gravity flow column. The resin was rinsed thrice with lysis buffer. Proteins bound to the resin were eluted with 4x 12.5 mL elution buffer (50 mM HEPES/NaOH pH 7.4, 500 mM NaCl, 300 mM imidazole, 0.5 mM TCEP) and diluted with 50 mL no salt buffer (20 mM HEPES/NaOH pH 7.4, 0.5 mM TCEP). The next purification step was performed using an AKTAprime system combined with a 5 ml SP Sepharose column. After equilibrating the column with no salt buffer, the sample was loaded. The column was rinsed with 10% high salt buffer (20 mM HEPES/NaOH pH 7.4, 2.5 M NaCl, 0.5 mM TCEP). The protein was eluted by a linear gradient of 10-100% high salt buffer in 100 mL. Fractions containing protein were pooled, combined with recombinant TEV protease (mass ratio 1:25) and incubated with rotation (over night, 4°C). Following this, 5 mL Ni Sepharose was added, and the suspension was loaded onto a gravity flow column. The flowthrough was collected and concentrated to 5.2 mL. Finally, the protein was polished by gel filtration using an AKTAxpress system with an S200 16/600 column and GF buffer (20 mM HEPES/NaOH pH 7.4, 500 mM NaCl, 0.5 mM TCEP). Fractions containing protein were pooled, concentrated and stored at -80°C.

Extraction

Buffers
Procedure
Exponentially growing Sf9 cells (2x 106 cells/mL) were infected with high titre baculovirus stock (1:65) and incubated in shaker flasks (60 hours, 90 rpm, 27°C). Following this, the cell suspensions were centrifuged (15 minutes, 800x g, 4°C), and the cell pellets resuspended in PBS. After another centrifugation (15 minutes, 800x g, 4°C), the cell pellets were stored at -80°C.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals grew from a 1:2 ratio of protein solution (6.7 mg/mL) and precipitant solution (1.7 M (NH4)2SO4, 0.1 M citrate pH 5.8, 0.2 M Na/K tartrate), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 25% ethylene glycol and flash frozen in liquid nitrogen. Data was collected at Diamond, beamline I02.
Data Processing: