HPGD

PDB:2GDZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AU63-G12; BC018986
Entry Clone Source: Synthetic
SGC Clone Accession:
Tag:C-terminal fusion: gskenlyfq*ghhhhhh
Host:BL21(DE3)-R3

Construct

Prelude:
Sequence:

MAHMVNGKVALVTGAAQGIGRAFAEALLLKGAKVALVDWNLEAGVQCKAALHEQFEPQKTLFIQCDVADQQQLRDTFRKVVDHFGRL
DILVNNAGVNNEKNWEKTLQINLVSVISGTYLGLDYMSKQNGGEGGIIINMSSLAGLMPVAQQPVYCASKHGIVGFTRSAALAANLM
NSGVRLNAICPGFVNTAILESIEKEENMGQYIEYKDHIKDMIKYYGILDPPLIANGLITLIEDDALNGAIMKITTSKGIHFQDYgsk
enlyfqghhhhhh

Vector:p15

Growth

Medium:
Antibiotics:
Procedure:TB. 10ml of overnight culture was added into 1L TB with 50 μg/ml of Ampicillin (total 6L). The cells were cultured at 37°C until the OD reached 1.405 and then decreased the temperature to 18°C. IPTG was added at 0.5mM (final concentration) and kept the culture at 18°C for overnight

Purification

Procedure
Column 1: Ni-NTA:
The column was packed by 4 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The column was washed with 50 ml of binding buffer and then 50 ml of washing buffer. The protein was eluted with 12 ml of elution buffer and collected by 1.5 ml fractions.

Column 2: Superdex 200 Hiload 16 60
AKTA Purifier was used. After running the SDS gel, 11 fractions were combined together for

TEV cleavage.

Column 3: Ni-NTA
His-tag was cleaved by TEV protease. 200 µl of TEV protease were added into the the sample after gel filtration. The sample was incubated at 4°C overnight.The sample was loaded onto the column (packed from 0.4 ml 0f Ni-NTA slurry). The flow through was collected and the column was then washed with 5 ml of the buffer (also collected).

Extraction

Procedure
The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37505 g. The supernatant was kept for further purification
Concentration:17mg/ml
Ligand
MassSpec:29019
Crystallization: Crystals were grown by vapour diffusion at 4°C in 200 nl sitting drops. The drops were prepared by mixing 100 nl of protein solution and 100 nl of precipitant consisting of 30% PEG 1K.
Crystals were transferred to a cryo-protectant consisting of 20% glycerol, 80 % well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.65 Å; X-ray source: Rotating anode, Rigaku FR-E superbright
Data Processing: