PTPN9: Human protein tyrosine phosphatase, non-receptor type 9

PDB:2PA5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:3895727
Entry Clone Source:MGC
SGC Clone Accession:
Tag:
Host: Phage-resistant derivative of BL21(DE3)

Construct

Prelude:NON-cleavable C-terminal His 6 tag
Sequence:

MSVHVPGPHAMTIQELVDYVNARQKQGIY EEYEDIRRENPVGTFHCSMSPGNLEKNRY GDVPCLDQTRVKLTKRSGHTQTDYINA
SF MDGYKQKNAYIGTQGPLENTYRDFWLMVW EQKVLVIVMTTRFEEGGRRKCGQYWPLEK DSRIRFGFLTVTNLGVENMNHYKK
TTLEI HNTEERQKRQVTHFQFLSWPDYGVPSSAA SLIDFLRVVRNQQSLAVSNMGARSKGQCP EPPIVVHCSAGIGRTGTFCSL
DICLAQLE ELGTLNVFQTVSRMRTQRAFSIQTPEQYY FCYKAILEFAEKEGMVSahhhhhh

Vector:pNIC-CH

Growth

Medium:LB
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 litre of LB containing 50 µg/ml kanamycin. Cultures were grown at 37degC until the OD600 reached ~0.3 then the temperature was adjusted to 18degC. Expression was induced for 4 hours using 1 mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen.

Purification

Procedure

Extraction

Procedure
Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.
Concentration:
Ligand
MassSpec:LC- ESI -MS TOF indicated a loss of 130 a.m.u. corresponding to the proteolytic cleavage of the initial methionine. Expected mass : 36179; Observed mass: 36049.
Crystallization: Crystals were grown at 20°C in 200 nl sitting drops mixing 100 nl of protein with 100 nl of a solution containing 25% PEG-3350, 0.2 M potassium thiocyanate, 10% ethylene glycol, 0.1 M Bis-Tris propane pH 6.15. The crystals were cryo-protected using 20% ethylene glycol which was added to the drop 30 seconds prior to mounting and flash freezing in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: