PTPRG D1-D2: Human Protein Tyrosine Phosphatase Receptor type Gamma D1-D2 domain

PDB:2NLK

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|18860898; NM_002841
Entry Clone Source: FivePrime
SGC Clone Accession:
Tag:C-terminal his6 tag
Host:BL21 (DE3) Rosetta R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

MAIPVKQFVKHIGELYSNNQHGFSEDFEE VQRCTADMNITAEHSNHPENKHKNRYINI LAYDHSRVKLRPLPGKDSKHSDYINAN
YV DGYNKAKAYIATQGPLKSTFEDFWRMIWE QNTGIIVMITNLVEKGRRKCDQYWPTENS EEYGNIIVTLKSTKIHACYTVRRF
SIRNT KVKKGQKGNPKGRQNERVVIQYHYTQWPD MGVPEYALPVLTFVRRSSAARMPETGPVL VHCSAGVGRTGTYIVIDSMLQ
QIKDKSTV NVLGFLKHIRTQRNYLVQTEEQYIFIHDA LLEAILGKETEVSSNQLHSYVNSILIPGV GGKTRLEKQFKLVTQCNA
KYVECFSAQKE CNKEKNRNSSVVPSERARVGLAPLPGMKG TDYINASYIMGYYRSNEFIITQHPLPHTT KDFWRMIWDHNAQII
VMLPDNQSLAEDEF VYWPSREESMNCEAFTVTLISKDRLCLSN EEQIIIHDFILEATQDDYVLEVRHFQCPK WPNPDAPISSTF
ELINVIKEEALTRDGPT IVHDEYGAVSAGMLCALTTLSQQLENENA VDVFQVAKMINLMRPGVFTDIEQYQFIYK MLSLVSTKE
NGNGPMTVDKNGAVLIADES DPAESMESLVahhhhhh

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight was used to inoculate 1 l of TB medium containing 50 microgram/ml Kanamycine and 34 µg/ml Chloramphenicole. E .coli cells were grown in 2.5-L baffled flasks at 37 degrees until OD reached 2.0. The cells were cooled to 18°C and expression of PTPRG was induced adding 0.5 mM IPTG at an OD600 of 2.2

Purification

Procedure

Extraction

Procedure
50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, Complete® protease inhibitors (1 tablet/50 ml) and 15 units/ml Benzonase.
Frozen cell pellets were thawed at 37 degC and re-suspended in a total volume of 100 ml lysis buffer. The cells were disrupted by a high pressure cell disrupter (20 kpsi) followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40 000xg. The supernatant was further clarified by filtration (0.45 μm).
Concentration:
Ligand
MassSpec: One peak of 71542 Da was detected for PTPTGAp011, which are 134 Da lower than the expected mass of 71674 . The discrepancy in mass was interpreted as a loss of the N-terminal methionine during expression.
Crystallization: Crystals were grown by vapor diffusion at 4degC from a sitting drop consisting of 100 nl protein (12.8 mg/ml) and 50 nl well solution. The drop was equilibrated against well solution containing 10 % PEG 10K and 100 mM imidazole pH 8.0. The crystal was transferred to a cryoprotectant composed of 20% ethylene glycol before flash-cooling in liquid nitrogen .
NMR Spectroscopy:
Data Collection: Resolution: 2.4 Å diffraction data were collected using a Rigaku FRE X-ray generator equipped with a HTC image plate detector.
Data Processing: