

PTPRK

PDB:2C7S

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:gi|18860902

Entry Clone Source:Purely Proteins

SGC Clone Accession:

Tag:Tag sequence: mhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.

Host:BL21 (DE3)

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfq*smPAIRVADLLQHINLMKTSDSYGFKEEYESFFEGQSASWDVAKKDQNRANKRYGNIIAYDHSRV
ILQPVEDDPSSDYINANYIDGYQRPSHYIATQGPVHETVYDFWRMIWQEQSACIVMVTNLVEVGRVKCYKYWPDDTEVYGDFKVTCTV
EMEPLAEYVVRTFTLERRGYNEIREVKQFHFTGWPDHGVPYHATGLLSFIRRVKLSNPPSAGPIVVHCSAGAGRTGCYIVIDIMLDM
AEREGVVDIYNCVKALRSRRINMVQTEEQYIFIHDAILEACLCGETAIPVCEF*DSKGGYGSE

Vector:pLIC-SGC

Growth

Medium:

Antibiotics:

Procedure:1mL from a 10 mL LB overnight culture containing 50 Âµg/mL kanamycin was used to inoculate 1 liter of LB media containing 50 Âµg/mL kanamycin. Cultures were grown at 37Â°C until the OD600 reached ~0.3. After that the temperature was adjusted to 18Â°C.

Expression was induced for 4 hours using 1mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

Purification

Procedure

Column 1: Ni-affinity chromatography.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500mM NaCl, 5% Glycerol. Wash buffer: 50 mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol. Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 50 to 250mM Imidazole, 5% Glycerol.

Procedure: 5 mL of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 30 mL binding buffer. The lysate was applied to the column which was subsequently washed with 3 x 10 mL wash buffer. PTPRK was eluted by a step gradient generated by 5 mL portions of elution buffer with increasing concentration of imidazole (concentrations used: 50mM, 100mM, 250mM). The eluted protein fractions were collected and analyzed by SDS PAGE. After elution DTT was added to a final concentration of 10mM.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)

SEC -Buffers: 10 mM Hepes, pH 7.5, 25 mM NaCl.

Procedure: The fractions eluted of the Ni-affinity chromatography were pooled and concentrated to about 1 mL using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 1 mL/min. Eluted fractions were 95% pure as judged by SDS - PAGE.

Protein concentration: Centricon with a 10kDa cut off in SEC -buffer

Extraction

Procedure

Cell pellets were lysed using a high pressure cell disrupter. The lysate was centrifuged at 50,000 rpm for 40 minutes and the supernatant collected for purification.

Concentration:

MassSpec:

Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/mL by mixing 100nl of the concentrated protein with 100nl of a well solution containing 0.20 M NaNO₃, 20.0% PEG 3350, 10.0% Ethylene glycole.

NMR Spectroscopy:

Data Collection: Resolution: 1.9 Å; Crystals were cryo-protected using the well solution and flash frozen in liquid nitrogen. X-ray source: Diffraction data were collected at the SLS beamLine X10 at a single wavelength (0.99 nm).

Data Processing: