NHERF-2: Human solute carrier family 9 isoform 3 regulator 2 (SLC 9A3R2)

PDB:2HE4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SLC 9A3R2A_s001
Entry Clone Source: Origene
SGC Clone Accession:
Tag:
Host:BL-21(DE3)R3 phage resistant

Construct

Prelude:N-terminal hexahistidine tag before TEV cleavage site; C-terminal PDZ recognition motif (STRL).
Sequence:

smLRPRLCHLRKGPQGYGFNLHSDKSRPG QYIRSVDPGSPAARSGLRAQDRLIEVNGQ NVEGLRHAEVVASIKAREDEARLLVVD PSTRL

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformed 90 micrL competent BL-21 (DE3) phage resistant cells with 2 µl of the plasmid DNA and plated out onto LB plate plus 50 µg/ml kanamycin and 34µg/ml chloramphenicol. The next two colonies were picked out into 50 ml LB + 50 µg/ml kanamycin + 34µg/ml chloramphenicol. 13 ml of the culture was used to innoculate 1 litre of TB + 50 µg/ml kanamycin. After 3.5 hrs of growth at 37°C the incubator temperature was dropped to 25°C. Growth was continued for a further 4 hrs before the OD600 reached a value of 1.8 at which point protein production was induced with the addition of 0.1 mM IPTG. The next morning cells were harvested by centrifugation at 4000 rpm for 15 minutes and resuspended in Lysis Buffer to a final volume of 30 mls before storage in the -80°C freezer. Lysis Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5 % glycerol, 5 mM imidazole pH 7.5, 0.5 mM TCEP.

Purification

Procedure
Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham)

Procedure: The cell extract was loaded on the column at 0.8 ml/min on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Affinity Binding buffer, 10 column volumes of Affinity wash buffer, the 10 column volumes of Affinity Wash Buffer II and SLC 9A3R2A eluted with Affinity elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration, Hiload 16/60, S75 16/60 - 120 ml.
The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions.

Enzymatic treatment : At this stage the purity of the protein was greater than 95 % based on SDS - PAGE analysis. The C-terminal hexahistidine tag was removed by TEV protease treatment. The TEV protease, a hexahistidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/ml. Add 30 m l of the TEV protease was added to each fraction and left at 4°C overnight. The following steps were carried out to remove the cleaved products and TEV protease. Place 300 m l of 50 % Ni2+-Sepharose agarose in a 1.5 ml eppendorf tube, add 1ml of 50 mM Tris pH 8, 150 mM NaCl mix, spin down and remove buffer. Repeat this resin wash step once. Add the TEV treated protein sample to the resin and mix for 30 min. Finally spin down resin and collect the supernatant which contains the cleaved SLC 9A3R2A. The sample was then concentrated to 2.4 mg/ml using a 10 kD cutoff spin concentrator.

Extraction

Procedure
After thawing the resuspended cells were lysed by passing through an Emulsiflex C5 high pressure homogeniser, 3 cycles, bringing the final volume to 90 ml. PEI (stock 5 %) was added to the homogenate to a final concentration of 0.15 %. The cell debris, nuclei and DNA were spun down at 16,500rpm for 45 min (rotor JA 17). The supernatant was collected.
Concentration:
Ligand
MassSpec: After His-Tag removal - Expected MWt.: 9971.2; Measured MWt.:9913.8.

NOTE: In the electron density maps the complete side chain density for Asp229 is missing. It appears that this residue has been mutated to a Gly. The difference in the observed to the expected molecular weight (52.6 daltons) corresponds with the difference in molecular weight between a Gly and Asp side chain.
Crystallization: Crystals grew at 20 degC from a 2:1 ratio mix of SLC 9A3R2A-to-reservoir (40 % PEG 300, 0.1 M citrate/phosphate buffer pH 4.2).
NMR Spectroscopy:
Data Collection:
Data Processing: