NhaA - Na(+)/H(+) antiporter # PL032

Product specification

Acronym: NhaA
Origin species : Bacterial
Protein reference : P13738 (UniProtKB)
NP_414560 (GenBank)
Family: Transporter

Expression system: E.coli based CFPS
Format: Proteoliposomes
Protein sequence: Met1 – Val388
Tag : 6xHis tag (N-ter)
Cleavage site: Factor Xa
Product MW: 43.9 kDa
Application: Drug screening & discovery, antibody development, structural biology

Product description

Na+/H+ antiporter that extrudes sodium in exchange for external protons. Catalyzes the exchange of 2 H+ per Na+. Can mediate sodium uptake when a transmembrane pH gradient is applied. Active at alkaline pH. Activity is strongly down-regulated below pH 6.5.

Recombinant protein sequence

His tag – factor X cleavage site –
MKHLHRFFSSDASGGIILIIAAILAMIMANSGATSGWYHDFLETPVQLRVGSLEINKNMLLWINDALMAVFFLLVGLEVKRELMQGS
LASLRQAAFPVIAAIGGMIVPALLYLAFNYADPITREGWAIPAATDIAFALGVLALLGSRVPLALKIFLMALAIIDDLGAIIIIALFYTN
DLSMASLGVAAVAIAVLAVLNLCGARRTGVYILVGVVLWTAVLKSGVHATLAGVIVGFFIPLKEKHGRSPAKRLEHVLHPWVAYLIL
PLFAFANAGVSLQGVTLDGLTSILPLGIIAGLLIGKPLGISLFCWLALRLKLAHLPEGTTYQQIMVVGILCGIGFTMSIFIASLAFGSVD
PELINWAKLGILVGSISSAVIGYSWLRVRLRPSV

Quality analysis

Purity:

Liposomes are directly incorporated into the Cell-Free reaction, thus, some impurities from the *E.coli* lysate might be present in the proteoliposomes.

A negative control (proteoliposomes without the protein of interest) can be provided (useful for screening, immunization…).

The purity can be improved by protein expression in detergent and relipidation after purification step(s).

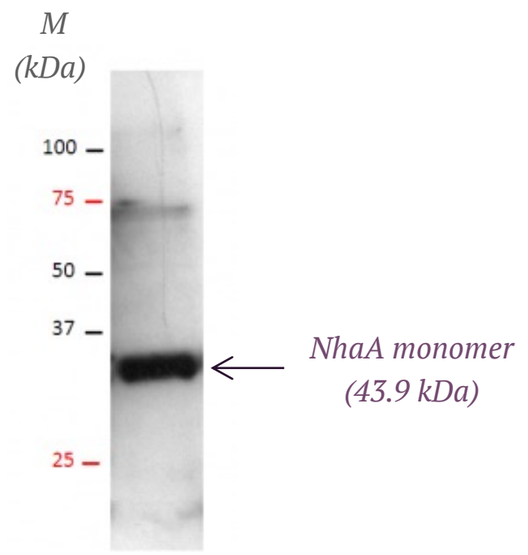


Fig.1: *Identification of NhaA in the proteoliposomes by Western blot using an anti-6xHis antibody*

Purification procedure: NhaA proteoliposomes are purified on a sucrose gradient.

NB : Migration of membrane proteins on SDS-PAGE can result in « gel shifting » due to the presence of hairpins (helix-loop-helix)[1-3].

References :

1 – Rath A., et al., Detergent binding explains anomalous SD-PAGE migration of membrane proteins PNAS, 2009 Feb 10, vol. 106

2 – Rath A., et al., Acrylamide concentration determines the direction and magnitude of helical membrane protein gel shifts, PNAS, 2013 Sep 24, 110(39)

3 – Rath A., et al., Correction factors for membrane protein molecular weight readouts on sodium dodecyl sulfate-polyacrilamide gel electrophoresis, Anal. Biochem., 2013 Mar 1, 434(1)

Assessment of functionality

Cell-free expression systems provide a real alternative for membrane protein expression, enabling the study of structure and function of membrane proteins.

Methods: Implantable biofuel cell

Results:

The proteoliposomes were added to the tethered membrane at the time indicated by the arrow labeled “a”. The conductance of the lipid membrane was measured continuously by AC impedance spectroscopy. The proteoliposomes were stably incorporated into the membrane after about 20 minutes, as indicated by the stabilization in the membrane conductance (bar labeled “incorporation”). It is important to note that the NhaA-proteoliposomes caused increased conductance during the incorporation phase, which was due to the functional NhaA protein in the proteoliposomes compared to the empty proteoliposomes. During the “function” phase of the measurements, the addition of 80 µM NaCl (arrow labeled “b”) increased the conductance of the lipid membrane that contained NhaA (solid trace) but had no effect on the control membrane that did not contain NhaA. Further addition of 160 µM NaCl (arrow labeled “c”) did not further enhance the conduction of NhaA. The response of the NhaA protein to transport Na+ and hence increase of the membrane conductance is evidence that the co-transport protein incorporates in the lipid bilayer and functions properly.

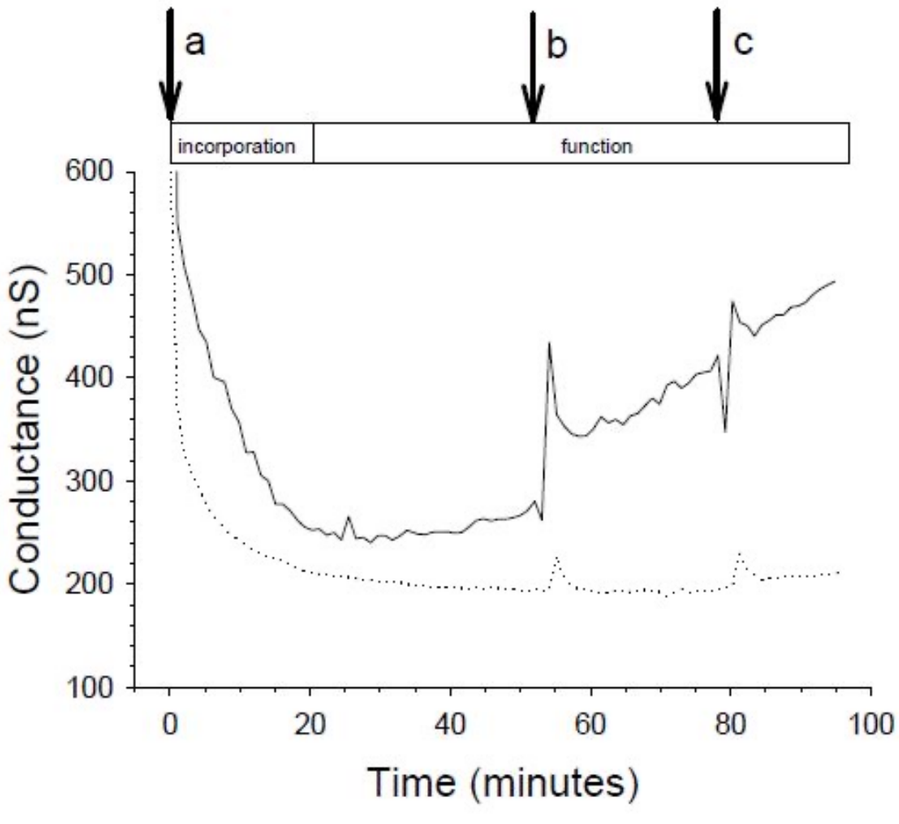


Fig.2: *Functional Incorporation of a Na+/H+ Co-transport Membrane Protein into a Lipid Bilayer. Incorporation of proteoliposomes that contain NhaA protein in a tethered lipid bilayer (solid trace). The control condition of empty proteoliposomes is indicated by the dotted trace.*

Formulation

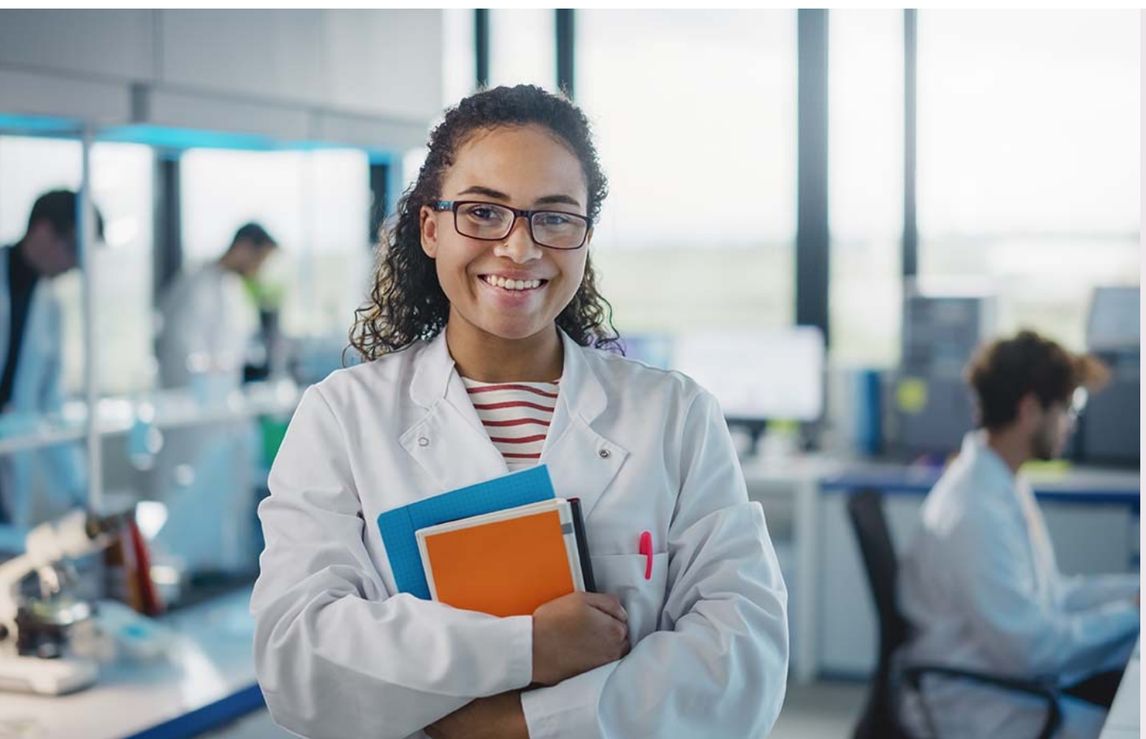
Buffer: Available in Hepes 50 mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage.
Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes: 10 µg, 50 µg, 100 µg, customized quantity on request.



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