

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

### Product specification

**Acronym:** NhaA

**Class:** Transporter

**Origin:** Bacterial

**Molecular weight:** 42 kDa

**Application:** Screening & Display Technologies

**Purity:** >40%

**Activity:** Proven

**Length:** Full Length

**TMD:** 12

**Biological function:** Exchange Na<sup>+</sup>/H<sup>+</sup>

### Product description

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

**Protein Source:** NhaA wild type protein

*Fig. 1: AA sequence of NhaA protein*

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10      20      30      40      50
MKHLHRRFFSS DASGGIILII AAILAMIMAN SGATS6WYHD FLETPVQLRV
60      70      80      90     100
GSLEINKNML LWINDALMAV FLLVGLVK RELMQGSLAS LRQAAPVIA
110     120     130     140     150
AIGGMIVPAL LYLAIFYADP ITREGWAIPA ATDIAFALGV LALLGSRVPL
160     170     180     190     200
ALKIFLMALA IIDDLGAIII IALFYTNDLS PASLGVAAVA IAVLAVLNLC
210     220     230     240     250
GARRTGVYIL VGVVLWTAVL KSGVHATLAG VIVGFFIPLK EKHGRSPAQR
260     270     280     290     300
LEHVLHPWA YLILPLFAFA NAGVSLOGVT LDGLTSILPL GIIAGLLIGK
310     320     330     340     350
PLGISLFCWL ALRLKLAHLP EGTTYQQIMV VGILCGIGFT MSIFIASLAF
360     370     380
GSVDEPINW AKLGILVGSV SSAVIGYSWL RVRLRPSV

```

**Affinity Tag:** Histidine tag fused to the N-terminal end of the protein.

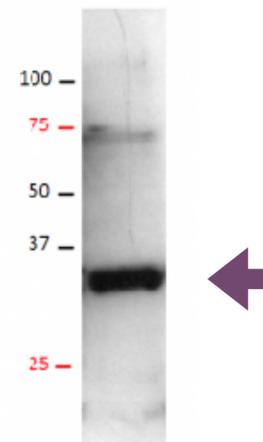
**Production conditions:** NhaA is expressed in a cell-free expression system in the presence of lipid vesicles. 100 µg can be produced and qualified in about 1 week

## Quality analysis

**Purity:** Typically >40% as determined by SDS-Page and Coomassie Blue staining.

**Purification procedure:** As standard, NhaA proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.

*Fig.2: Proteoliposome NhaA after purification (Western blot identification).*



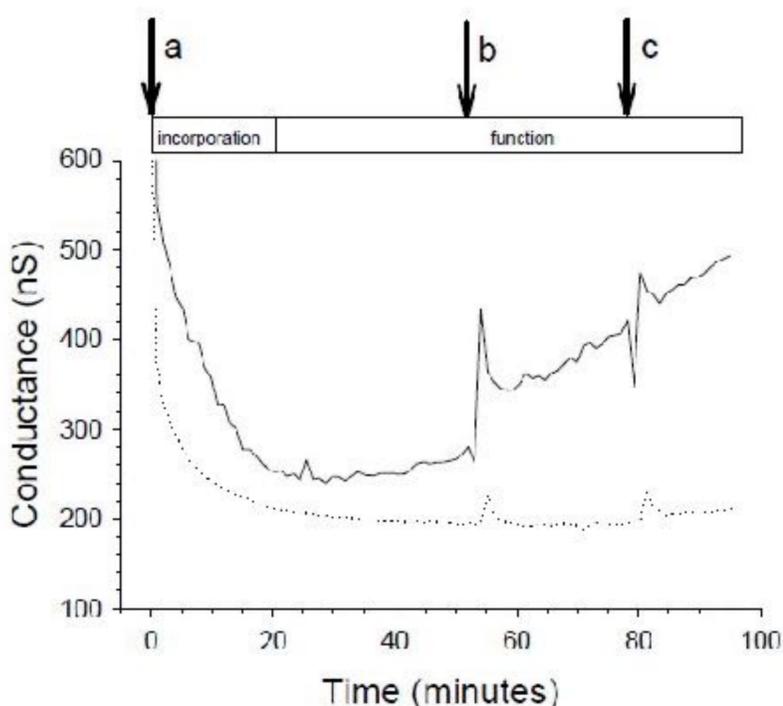
## Assessment of functionality

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 incorporated stably 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 $\mu$ M 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 $\mu$ M NaCl (arrow labeled “c”) did not further enhance the conduction of NhaA. The response of the NhaA protein to transport Na<sup>+</sup> and hence increase the membrane conductance is evidence that the co-transport protein incorporates in the lipid bilayer and functions properly.



*Fig.3: Functional Incorporation of a Na<sup>+</sup>/H<sup>+</sup> 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

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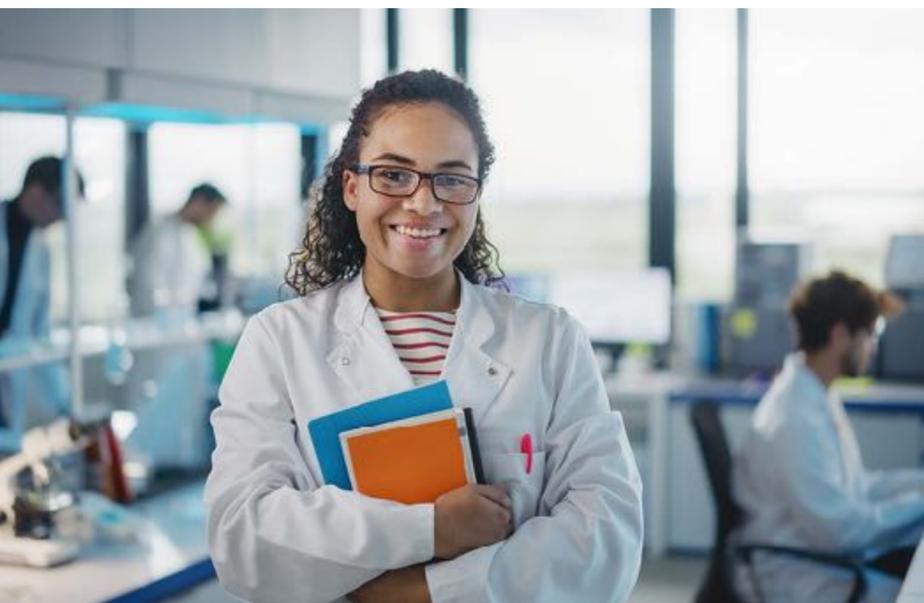
**Buffer:** Available in Tris 50mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

**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:** 10µg, 20µg, 100 µg, 200 µg, 500 µg, bulk



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