

# Na<sup>+</sup>/K<sup>+</sup>-ATPase $\alpha$ 2 siRNA (h): sc-42660

## BACKGROUND

The ubiquitously expressed sodium/potassium-ATPase (Na<sup>+</sup>/K<sup>+</sup>-ATPase) exists as an oligomeric plasma membrane complex that couples the hydrolysis of one molecule of ATP to the importation of three Na<sup>+</sup> ions and two K<sup>+</sup> ions against their respective electrochemical gradients. As a member of the P-type family of ion motives, Na<sup>+</sup>/K<sup>+</sup>-ATPase plays a critical role in maintaining cellular volume, resting membrane potential and Na<sup>+</sup>-coupled solute transport. Multiple isoforms of three subunits,  $\alpha$ ,  $\beta$  and  $\gamma$ , comprise the Na<sup>+</sup>/K<sup>+</sup>-ATPase oligomer. The  $\alpha$  subunit contains the binding sites for ATP and the cations, while the glycosylated  $\beta$  subunit ensures correct folding and membrane insertion of the  $\alpha$  subunits. The small  $\gamma$  subunit co-localizes with the  $\alpha$  subunit in nephron segments, where it increases the affinity of the Na<sup>+</sup>/K<sup>+</sup>-ATPase for ATP. While the  $\gamma$  subunit is not essential for normal activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase, the  $\beta$  subunit is.

## REFERENCES

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3. McDonough, A.A., et al. 1990. The sodium pump needs its  $\beta$  subunit. *FASEB J.* 4: 1598-1605.
4. Pedemonte, C.H., et al. 1990. Chemical modification as an approach to elucidation of sodium pump structure-function relations. *Am. J. Physiol.* 258: C1-C23.
5. Mercer, R.W., et al. 1993. Molecular cloning and immunological characterization of the  $\gamma$ -polypeptide, a small protein associated with Na,K-ATPase. *J. Cell Biol.* 121: 579-586.
6. DeTomaso, A.W., et al. 1993. Expression, targeting, and assembly of functional Na,K-ATPase polypeptides in baculovirus-infected insect cells. *J. Biol. Chem.* 268: 1470-1478.
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## CHROMOSOMAL LOCATION

Genetic locus: ATP1A2 (human) mapping to 1q23.2.

## PRODUCT

Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 shRNA Plasmid (h): sc-42660-SH and Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 shRNA (h) Lentiviral Particles: sc-42660-V as alternate gene silencing products.

For independent verification of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42660A, sc-42660B and sc-42660C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 siRNA (h) is recommended for the inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 expression in human cells.

## SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10  $\mu$ M in 66  $\mu$ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 (H-3): sc-48345 is recommended as a control antibody for monitoring of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 gene expression knockdown using RT-PCR Primer: Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 2 (h)-PR: sc-42660-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.