# SANTA CRUZ BIOTECHNOLOGY, INC.

# Na<sup>+</sup>/K<sup>+</sup>-ATPase $\alpha$ 3 siRNA (h): sc-36012



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# BACKGROUND

The ubiquitously expressed sodium/potassium-ATPase (Na+/K+-ATPase) exists as a oligomeric plasma membrane complex that couples the hydrolysis of one molecule of ATP to the importation of three Na+ ions and two K+ ions against their respective electrochemical gradients. As a member of the P-type family of ion motives, Na+/K+-ATPase plays a critical role in maintaining cellular volume, resting membrane potential and Na+-coupled solute transport. Multiple isoforms of three subunits,  $\alpha$ ,  $\beta$  and  $\gamma$ , comprise the Na+/K+-ATPase oligomer. The  $\alpha$  subunit contains the binding sites for ATP and the cations; 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 Na+/K+-ATPase for ATP. The  $\beta$  subunit, but not the  $\gamma$  subunit, is essential for normal activity of Na+/K+-ATPase.

### REFERENCES

- Hardwicke, P.M., et al. 1981. A proteolipid associated with Na,K-ATPase is not essential for ATPase activity. Biochem. Biophys. Res. Commun. 102: 250-257.
- 2. Ackermann, U., et al. 1990. Mutual dependence of Na,K-ATPase  $\alpha$  and  $\beta$ -subunits for correct post-translational processing and intracellular transport. FEBS Lett. 269: 105-108.
- 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.

#### CHROMOSOMAL LOCATION

Genetic locus: ATP1A3 (human) mapping to 19q13.2.

# PRODUCT

Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 3 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  $\alpha$ 3 shRNA Plasmid (h): sc-36012-SH and Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 3 shRNA (h) Lentiviral Particles: sc-36012-V as alternate gene silencing products.

For independent verification of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 3 (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-36012A, sc-36012B and sc-36012C.

#### 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+/K+-ATPase  $\alpha 3$  siRNA (h) is recommended for the inhibition of Na+/K+-ATPase  $\alpha 3$  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+/K+-ATPase  $\alpha$ 3 (H-4): sc-365744 is recommended as a control antibody for monitoring of Na+/K+-ATPase  $\alpha$ 3 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<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

# **RT-PCR REAGENTS**

Semi-quantitative RT-PCR may be performed to monitor Na+/K+-ATPase  $\alpha$ 3 gene expression knockdown using RT-PCR Primer: Na+/K+-ATPase  $\alpha$ 3 (h)-PR: sc-36012-PR (20 µl, 461 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

- 1. Xie, C.M., et al. 2013. Cardiac glycosides block cancer growth through HIF-1 $\alpha$  and NF $\kappa$ B-mediated Plk1. Carcinogenesis 34: 1870-1880.
- Xie, C.M., et al. 2018. Cardiac glycoside bufalin blocks cancer cell growth by inhibition of Aurora A and Aurora B activation via PI3K-Akt pathway. Oncotarget 9: 13783-13795.

## **RESEARCH USE**

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

#### PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.