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Na⁺/K⁺-ATPase α 2 siRNA (m): sc-42661

BACKGROUND

The ubiquitously expressed sodium/potassium-ATPase (Na⁺/K⁺-ATPase) exists as an 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, α , β and γ , comprise the Na⁺/K⁺-ATPase oligomer. The α subunit contains the binding sites for ATP and the cations, while the glycosylated β subunit ensures correct folding and membrane insertion of the α subunits. The small γ subunit co-localizes with the α subunit in nephron segments, where it increases the affinity of the Na⁺/K⁺-ATPase for ATP. While the γ subunit is not essential for normal activity of Na⁺/K⁺-ATPase, the β subunit is.

REFERENCES

1. 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 α - and β -subunits for correct post-translational processing and intracellular transport. *FEBS Lett.* 269: 105-108.
3. 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: Atp1a2 (mouse) mapping to 1 H3.

PRODUCT

Na⁺/K⁺-ATPase α 2 siRNA (m) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Na⁺/K⁺-ATPase α 2 shRNA Plasmid (m): sc-42661-SH and Na⁺/K⁺-ATPase α 2 shRNA (m) Lentiviral Particles: sc-42661-V as alternate gene silencing products.

For independent verification of Na⁺/K⁺-ATPase α 2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42661A, sc-42661B and sc-42661C.

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

APPLICATIONS

Na⁺/K⁺-ATPase α 2 siRNA (m) is recommended for the inhibition of Na⁺/K⁺-ATPase α 2 expression in mouse 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 μ M in 66 μ 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 α 2 (H-3): sc-48345 is recommended as a control antibody for monitoring of Na⁺/K⁺-ATPase α 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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⁺/K⁺-ATPase α 2 gene expression knockdown using RT-PCR Primer: Na⁺/K⁺-ATPase α 2 (m)-PR: sc-42661-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kinoshita, P.F., et al. 2017. α 2 Na⁺,K⁺-ATPase silencing induces loss of inflammatory response and ouabain protection in glial cells. *Sci. Rep.* 7: 4894.

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.