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Na⁺/K⁺-ATPase α shRNA (h) Lentiviral Particles: sc-43956-V

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, α , β and γ , comprise the Na⁺/K⁺-ATPase oligomer. The α subunit contains the binding sites for ATP and the cations; 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 Na⁺/K⁺-ATPase for ATP. The β subunit, but not the γ subunit, is essential for normal activity of Na⁺/K⁺-ATPase.

REFERENCES

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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.
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PRODUCT

Na⁺/K⁺-ATPase α shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Na⁺/K⁺-ATPase α siRNA (h): sc-43956 and Na⁺/K⁺-ATPase α shRNA Plasmid (h): sc-43956-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

Na⁺/K⁺-ATPase α shRNA (h) Lentiviral Particles is recommended for the inhibition of Na⁺/K⁺-ATPase α expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Na⁺/K⁺-ATPase α (M7-PB-E9): sc-58628 is recommended as a control antibody for monitoring of Na⁺/K⁺-ATPase α 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Na⁺/K⁺-ATPase α gene expression knockdown using RT-PCR Primer: Na⁺/K⁺-ATPase α (h)-PR: sc-43956-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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