SANTA CRUZ BIOTECHNOLOGY, INC.

AE2 siRNA (h): sc-60056

BACKGROUND

Primary canalicular bile undergoes a process of fluidization and alkalinization along the biliary tract that is influenced by several factors, including hormones, innervation/neuropeptides and biliary constituents. The excretion of bicarbonate at both the canaliculi and the bile ducts is an important contributor to the generation of bile-salt independent flow. Bicarbonate is secreted from hepatocytes and cholangiocytes through parallel mechanisms, which involve chloride efflux through activation of chloride channels and further bicarbonate secretion via AE2 (also designated SLC4A2)-mediated chloride/bicarbonate exchange. The AE2 protein regulates pH, chloride concentration, cell volume and transepithelial ion transport in many tissues. Gene silencing of AE2 causes a marked inhibition of unstimulated and secretin-stimulated chloride/bicarbonate exchange, which maintains the bile acid pool that is crucial for secretin to induce bicarbonate-rich choleresis.

REFERENCES

1. Eladari, D., et al. 1998. Functional and molecular characterization of luminal and basolateral Cl^-/HCO^- exchangers of rat thick limbs. Am. J. Physiol. 275: F334-F342.
2. Aranda, V., et al. 2004. Shared apical sorting of anion exchanger isoforms AE2a, AE2b1, and AE2b2 in primary hepatocytes. Biochem. Biophys. Res. Commun. 319: 1040-1046.
3. Gawenis, L.R., et al. 2004. Mice with a targeted disruption of the AE2 Cl^-/HCO_3^- exchanger are achlorhydric. J. Biol. Chem. 279: 30531-30539.
4. Stewart, A.K., et al. 2004. Acute pH-dependent regulation of AE2-mediated anion exchange involves discrete local surfaces of the NH_2-terminal cytoplasmic domain. J. Biol. Chem. 279: 52664-52676.
5. Banales, J.M., et al. 2006. Bicarbonate-rich choleresis induced by secretin in normal rat is taurocholate-dependent and involves AE2 anion exchanger. Hepatology 43: 266-275.
6. Banales, J.M., et al. 2006. Cholangiocyte anion exchange and biliary bicarbonate excretion. World J. Gastroenterol. 12: 3496-3511.
7. Kurschat, C.E., et al. 2006. Alkaline-shifted pHo sensitivity of AE2c1-mediated anion exchange reveals novel regulatory determinants in the AE2 N-terminal cytoplasmic domain. J. Biol. Chem. 281: 1885-1896.

CHROMOSOMAL LOCATION

Genetic locus: SLC4A2 (human) mapping to 7q36.1.

PRODUCT

AE2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AE2 shRNA Plasmid (h): sc-60056-SH and AE2 shRNA (h) Lentiviral Particles: sc-60056-V as alternate gene silencing products.

For independent verification of AE2 (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-60056A, sc-60056B and sc-60056C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AE2 siRNA (h) is recommended for the inhibition of AE2 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 µM in 66 µl. 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

AE2 (D-3): sc-376632 is recommended as a control antibody for monitoring of AE2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 AE2 gene expression knockdown using RT-PCR Primer: AE2 (h)-PR: sc-60056-PR (20 µl, 422 bp). 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 for detailed protocols and support products.