

UPIIIa siRNA (h): sc-41096

BACKGROUND

The asymmetric unit membrane (AUM) forms numerous plaques, which cover the apical surface of the urothelium. These plaques are thought to strengthen the urothelium and reduce the risk of rupturing during ladder distention. They are composed of four major integral membrane proteins called uroplakins (UP). The uroplakin family comprises UPIa, UPIb, UPII, and UPIII. Family members are conserved among several species, including human, mouse, rat, rabbit, canine, porcine and ovine. UPIa and UPIb form tightly packed structures with UPII and UPIII, respectively. This pairing is required for normal urothelial plaque formation and is regulated by proteolytic processing of the uroplakin proteins. Uroplakins are expressed in normal urothelium and are used as specific markers of urothelial differentiation. They are also expressed in a majority of transitional cell carcinomas of the bladder (TCCs), which make the uroplakins a useful marker for detecting bladder cancer metastasis and for staging and monitoring chemotherapeutic response.

REFERENCES

1. Lin, J.H., et al. 1994. Precursor sequence, processing, and urothelium-specific expression of a major 15-kDa protein subunit of asymmetric unit membrane. *J. Biol. Chem.* 269: 1775-1784.
2. Wu, X.R., et al. 1994. Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. *J. Biol. Chem.* 269: 13716-13724.
3. Wu, X.R., et al. 1995. Selective interactions of UPIa and UPIb, two members of the transmembrane 4 superfamily, with distinct single transmembrane-domained proteins in differentiated urothelial cells. *J. Biol. Chem.* 270: 29752-29759.
4. Li, S.M., et al. 1999. Detection of circulating uroplakin-positive cells in patients with transitional cell carcinoma of the bladder. *J. Urol.* 162: 931-935.
5. Shapiro, E., et al. 2000. Uroplakin and androgen receptor expression in the human fetal genital tract: insights into the development of the vagina. *J. Urol.* 164: 1048-1051.
6. Hu, P., et al. 2000. Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. *J. Cell Biol.* 151: 961-972.

CHROMOSOMAL LOCATION

Genetic locus: UPK3A (human) mapping to 22q13.31.

PRODUCT

UPIIIa 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UPIIIa shRNA Plasmid (h): sc-41096-SH and UPIIIa shRNA (h) Lentiviral Particles: sc-41096-V as alternate gene silencing products.

For independent verification of UPIII (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-41096A, sc-41096B and sc-41096C.

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

UPIIIa siRNA (h) is recommended for the inhibition of UPIIIa 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 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

UPIIIa (C-6): sc-166808 is recommended as a control antibody for monitoring of UPIIIa 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 λ BP-HRP: sc-516132 or m-IgG λ BP-HRP (Cruz Marker): sc-516132-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 λ BP-FITC: sc-516185 or m-IgG λ BP-PE: sc-516186 (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 UPIIIa gene expression knockdown using RT-PCR Primer: UPIIIa (h)-PR: sc-41096-PR (20 μ 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 for detailed protocols and support products.