

SP-D siRNA (h): sc-36541

BACKGROUND

Pulmonary surfactant is primarily responsible for lowering the surface tension at the air-liquid interface in the alveoli, a process that is essential for normal respiration. Pulmonary surfactant is a mixture of phospholipids and proteins, including four distinct surfactant-associated proteins (SPs), SP-A, SP-B, SP-C and SP-D. SP-B and SP-C are predominantly hydrophobic proteins that associate with lipids to promote the absorption of surfactant phospholipids and to reduce the surface tension in the alveoli. SP-A and SP-D are large multimeric proteins belonging to the family of calcium-dependent lectins, designated collectins, which contribute to the innate immune system. Both SP-A and SP-D have been shown to protect against microbial challenge through binding to the lipid components of the bacterial cell wall and facilitating the rapid removal of microbes.

REFERENCES

1. Glasser, S.W., et al. 1990. Structure and expression of the pulmonary surfactant protein SP-C gene in the mouse. *J. Biol. Chem.* 265: 21986-21991.
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3. Johansson, J., et al. 1992. Human surfactant polypeptide SP-B. Disulfide bridges, C-terminal end and peptide analysis of the airway form. *FEBS Lett.* 301: 165-167.
4. Crouch, E., et al. 1993. Genomic organization of human surfactant protein D (SP-D). SP-D is encoded on chromosome 10q22.2-23.1. *J. Biol. Chem.* 268: 2976-2983.
5. Rooney, S.A., et al. 1994. Molecular and cellular processing of lung surfactant. *FASEB J.* 8: 957-967.
6. Johansson, J., et al. 1997. Molecular structures and interactions of pulmonary surfactant components. *Eur. J. Biochem.* 244: 675-693.
7. Reid, K.B. 1998. Functional roles of the lung surfactant proteins SP-A and SP-D in innate immunity. *Immunobiology* 199: 200-207.
8. Wert, S.E., et al. 2000. Increased metalloproteinase activity, oxidant production and emphysema in surfactant protein D gene-inactivated mice. *Proc. Natl. Acad. Sci. USA* 97: 5972-5977.

CHROMOSOMAL LOCATION

Genetic locus: SFTPD (human) mapping to 10q22.3.

PRODUCT

SP-D 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 SP-D shRNA Plasmid (h): sc-36541-SH and SP-D shRNA (h) Lentiviral Particles: sc-36541-V as alternate gene silencing products.

For independent verification of SP-D (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-36541A, sc-36541B and sc-36541C.

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

SP-D siRNA (h) is recommended for the inhibition of SP-D 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

SP-D (C-6): sc-25324 is recommended as a control antibody for monitoring of SP-D 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 MarkerTM 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 SP-D gene expression knockdown using RT-PCR Primer: SP-D (h)-PR: sc-36541-PR (20 μ l, 592 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.