

## DEF-3 siRNA (h): sc-106849

### BACKGROUND

DEF-3 and LUCA15 belong to an evolutionarily conserved family of RNA binding proteins and share similar expression patterns. Both DEF-3 and LUCA15 are highly expressed in adult heart and thymus as well as fetal kidney. Conversely, fetal thymus and adult kidney express very little DEF-3 and LUCA15. In the hemopoietic system of mice, the expression of DEF-3 is downregulated upon differentiation of progenitor cells into granulocytes but persists during macrophage development. Both DEF-3 and LUCA15 contain two zinc finger motifs, a bipartite nuclear signal and two RNA binding motifs. DEF-3 and LUCA15 are capable of specifically binding poly(G) RNA. The genes encoding human DEF-3 and LUCA15 map to 3p21.31, a region homozygously deleted in the small cell lung cancer cell line GLC20. The onset of lung cancer arises from mutations in dominant and recessive oncogenes, and chromosome 3p contains many of these recessive oncogenes.

### REFERENCES

1. Roche, J., et al. 1996. Distinct 3p21.3 deletions in lung cancer and identification of a new human semaphorin. *Oncogene* 12: 1289-1297.
2. Gure, A.O., et al. 1998. Human lung cancer antigens recognized by autologous antibodies: definition of a novel cDNA derived from the tumor suppressor gene locus on chromosome 3p21.3. *Cancer Res.* 58: 1034-1041.
3. Drabkin, H.A., et al. 1999. DEF-3(g16/NY-LU-12), an RNA binding protein from the 3p21.3 homozygous deletion region in SCLC. *Oncogene* 18: 2589-2597.
4. Hotfilder, M., et al. 1999. Def-2, -3, -6 and -8, novel mouse genes differentially expressed in the haemopoietic system. *Br. J. Haematol.* 106: 335-344.

### CHROMOSOMAL LOCATION

Genetic locus: RBM6 (human) mapping to 3p21.31.

### PRODUCT

DEF-3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DEF-3 shRNA Plasmid (h): sc-106849-SH and DEF-3 shRNA (h) Lentiviral Particles: sc-106849-V as alternate gene silencing products.

For independent verification of DEF-3 (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-106849A, sc-106849B and sc-106849C.

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

### APPLICATIONS

DEF-3 siRNA (h) is recommended for the inhibition of DEF-3 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  $\mu$ M in 66  $\mu$ 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

DEF-3 (D-3): sc-376201 is recommended as a control antibody for monitoring of DEF-3 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 DEF-3 gene expression knockdown using RT-PCR Primer: DEF-3 (h)-PR: sc-106849-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

1. Kim, H., et al. 2013. PDZK1 is a novel factor in breast cancer that is indirectly regulated by estrogen through IGF-1R and promotes estrogen-mediated growth. *Mol. Med.* 19: 253-262.

### RESEARCH USE

For research use only, not for use in diagnostic procedures.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.