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SANTA CRUZ BIOTECHNOLOGY, INC.

Dbs siRNA (h): sc-41728

BACKGROUND

The Dbl family act as guanine nucleotide exchange factors (GEFs) specific for Rho guanosine triphosphatases (GTPases). They regulate Rho GTPase function by stimulating formation of the active, GTP-bound state. All Dbl family members invariably possess a tandem domain structure, which consists of a Dbl homology (DH) catalytic domain followed by a Pleckstrin homology (PH) regulatory domain. Dbs (for Dbl's big sister), also known as Ost or MCF2L, differs from Dbl by the addition of an amino-terminal extension and a carboxy-terminal SH3 domain. Unlike Dbl, it also requires the presence of the PH domain for the intrinsic catalytic activity of the DH domain. The expression of Dbs is high in several tissues, including brain, and low in thymus and spleen. Dbs exhibits guanine nucleotide exchange activity for Rho A and Cdc42 to mediate growth deregulation. Dbs activity involves multiple signaling pathways that include activation of the Elk-1, Jun and NFκB transcription factors and stimulation of transcription from the cyclin D1 promoter.

REFERENCES

1. Whitehead, I., et al. 1995. Retroviral transduction and oncogenic selection of a cDNA encoding Dbs, a homolog of the Dbl guanine nucleotide exchange factor. Oncogene 10: 713-721.
2. Whitehead, I.P., et al. 1999. Dependence of Dbl and Dbs transformation on MEK and NFκB activation. Mol. Cell. Biol. 19: 7759-7770.
3. Rossman, K.L., et al. 2002. A crystallographic view of interactions between Dbs and Cdc42: PH domain-assisted guanine nucleotide exchange. EMBO J. 21: 1315-1326.
4. Rossman, K.L., et al. 2003. Multifunctional roles for the PH domain of Dbs in regulating Rho GTPase activation. J. Biol. Chem. 278: 18393-18400.

CHROMOSOMAL LOCATION

Genetic locus: MCF2L (human) mapping to 13q34.

PRODUCT

Dbs 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 Dbs shRNA Plasmid (h): sc-41728-SH and Dbs shRNA (h) Lentiviral Particles: sc-41728-V as alternate gene silencing products.

For independent verification of Dbs (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-41728A, sc-41728B and sc-41728C.

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

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

Dbs (C-7): sc-376400 is recommended as a control antibody for monitoring of Dbs 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 Dbs gene expression knockdown using RT-PCR Primer: Dbs (h)-PR: sc-41728-PR (20 µl, 489 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, Z., et al. 2009. The Rho-specific guanine nucleotide exchange factor Dbs regulates breast cancer cell migration. J. Biol. Chem. 284: 15771-15780.

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.