SANTA CRUZ BIOTECHNOLOGY, INC.

DBC-2 siRNA (h): sc-77501

BACKGROUND

The Rho subfamily of Ras-related GTPases controls multiple aspects of cell function, including cytoskeletal rearrangement, nuclear signaling and cell growth. DBC-2 (deleted in breast cancer 2 gene protein), also known as RHOBTB2 (Rho-related BTB domain-containing protein 2), is a 727 amino acid member of the RhoBTB subfamily of Rho GTPases. Members of the RhoBTB subfamily are evolutionarily conserved and are characterized by a proline-rich region, a GTPase domain and two tandem BTB repeats. Expressed ubiquitously with highest levels in neural tissue, heart, brain and fetal lung, DBC-2 contains two BTB (POZ) domains through which it may bind to and regulate the function of target proteins, such as CUL-3. Additionally, DBC-2 is thought to function as a regulator of cell cycle and apoptosis events. Under normal conditions, DBC-2 is thought to exhibit tumor suppressor activity. Mutations in the gene encoding DBC-2 are associated with breast cancer, suggesting that mutated DBC-2 may play a role in carcinogenesis.

REFERENCES

1. Ramos, S., Khademi, F., Somesh, B.P. and Rivero, F. 2002. Genomic organization and expression profile of the small GTPases of the RhoBTB family in human and mouse. Gene 298: 147-157.
2. Hamaguchi, M., Meth, J.L., von Klitzing, C., Wei, W., Esposito, D., Rodgers, L., Walsh, T., Welcsh, P., King, M.C. and Wigler, M.H. 2002. DBC-2, a candidate for a tumor suppressor gene involved in breast cancer. Proc. Natl. Acad. Sci. USA 99: 13647-13652.
3. Wilkins, A., Ping, Q. and Carpenter, C.L. 2004. RhoBTB2 is a substrate of the mammalian CUL-3 ubiquitin ligase complex. Genes Dev. 18: 856-861.
4. Siripurapu, V., Meth, J., Kobayashi, N. and Hamaguchi, M. 2005. DBC-2 significantly influences cell-cycle, apoptosis, cytoskeleton and membrane-trafficking pathways. J. Mol. Biol. 346: 83-89.
5. Chang, F.K., Sato, N., Kobayashi-Simorowski, N., Yoshihara, T., Meth, J.L. and Hamaguchi, M. 2006. DBC-2 is essential for transporting vesicular stomatitis virus glycoprotein. J. Mol. Biol. 364: 302-308.

CHROMOSOMAL LOCATION

Genetic locus: RHOBTB2 (human) mapping to 8p21.3.

PRODUCT

DBC-2 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 DBC-2 shRNA Plasmid (h): sc-77501-SH and DBC-2 shRNA (h) Lentiviral Particles: sc-77501-V as alternate gene silencing products.

For independent verification of DBC-2 (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-77501A, sc-77501B and sc-77501C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

DBC-2 siRNA (h) is recommended for the inhibition of DBC-2 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

DBC-2 (G-12): sc-398774 is recommended as a control antibody for monitoring of DBC-2 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 DBC-2 gene expression knockdown using RT-PCR Primer: DBC-2 (h)-PR: sc-77501-PR (20 µl). 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.