

BCAR3 siRNA (h): sc-60265

BACKGROUND

BCAR3 (breast cancer anti-estrogen resistance 3, also designated AND-34 in murine; a novel SH2-containing protein 2 or NSP2, and SH2D3B) overexpression is a characteristic of antiestrogen resistance in human ZR-75-1 breast cancer cells. The deduced 825-amino acid BCAR3 protein contains a Src homology 2 (SH2) domain and shares homology to yeast CDC48. A 3.4-kb BCAR3 transcript is present in heart, placenta, skeletal muscle, spleen, prostate, testis, ovary, small intestine, colon, fetal kidney, and tamoxifen-resistant cancer cell lines. BCAR3 acts as a regulator of R-Ras to mediate the level of Insulin receptor substrate 1 (IRS-1) in MCF-7 and ZR-75-1 breast cancer cell lines. BCAR3 also interacts with p130Cas to enhance Src activation and cell migration.

REFERENCES

1. van Agthoven, T., et al. 1998. Identification of BCAR3 by a random search for genes inv resistance of human breast cancer cells. *EMBO J.* 17: 2799-2808.
2. Gotoh, T., et al. 2000. p130Cas regulates nucleotide exchange factor. *J. Biol. Chem.* 275: 30118-30123.
3. Cai, D., et al. 2003. AND-34/BCAR3, a GDP exchange factor whose overexpression confers antiestrogen resistance, activates Rac, PAK1, and the cyclin D1 promoter. *Cancer Res.* 63: 6802-6808.
4. Riggins, R.B., et al. 2003. Synergistic promotion of c-Src activation and cell migration by Cas and AND-34/BCAR3. *J. Biol. Chem.* 278: 28264-28273.
5. Dorssers, L.C., et al. 2005. Breast cancer oestrogen independence mediated by BCAR1 or BCAR3 genes is transmitted through mechanisms distinct from the oestrogen receptor signalling pathway or the epidermal growth factor receptor signalling pathway. *Breast Cancer Res.* 7: R82-R92.
6. Yu, Y., et al. 2006. The R-Ras GTPase mediates cross talk between estrogen and Insulin signaling in breast cancer cells. *Mol. Cell. Biol.* 26: 6372-6380.

CHROMOSOMAL LOCATION

Genetic locus: BCAR3 (human) mapping to 1p22.1.

PRODUCT

BCAR3 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 BCAR3 shRNA Plasmid (h): sc-60265-SH and BCAR3 shRNA (h) Lentiviral Particles: sc-60265-V as alternate gene silencing products.

For independent verification of BCAR3 (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-60265A, sc-60265B and sc-60265C.

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 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

BCAR3 siRNA (h) is recommended for the inhibition of BCAR3 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

BCAR3 (3G4): sc-293346 is recommended as a control antibody for monitoring of BCAR3 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 Marker™ 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 BCAR3 gene expression knockdown using RT-PCR Primer: BCAR3 (h)-PR: sc-60265-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.