

BRAF35 siRNA (h): sc-37536

BACKGROUND

The breast cancer susceptibility gene (BRCA1) localizes to chromosome 17q. Mutations within this gene account for approximately 45% of families with high incidence of breast cancer and at least 80% of families with increased incidence of both early-onset breast cancer and ovarian cancer. A second breast cancer susceptibility gene, BRCA2, located on chromosome 13q13.3, also confers a high incidence of breast cancer, but unlike BRCA1, BRCA2 does not confer a substantially elevated risk of ovarian cancer. The BRCA2-associated factor 35 (BRAF35) protein forms a complex with BRCA2, which associates with condensed chromatin during Histone H3 phosphorylation. BRAF35 expression levels are highest in proliferating tissues and parallel BRCA2 expression patterns. The structure of BRAF35 includes a kinesin-like coiled-coil domain and a nonspecific DNA-binding HMG domain. The chromatin localization of BRAF35 and antibody microinjection studies indicate a role for the BRAF35/BRCA2 complex in cell cycle regulation.

REFERENCES

1. Hall, J.M., et al. 1990. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 250: 1684-1689.
2. Narod, S.A., et al. 1991. Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 338: 82-83.
3. Wooster, R., et al. 1994. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* 265: 2088-2090.
4. Futreal, P.A., et al. 1994. BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266: 120-122.
5. Marmorstein, L.Y., et al. 2001. A human BRCA2 complex containing a structural DNA binding component influences cell cycle progression. *Cell* 104: 247-257.
6. Hakimi, M.A., et al. 2002. A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. *Proc. Natl. Acad. Sci. USA* 99: 7420-7425.

CHROMOSOMAL LOCATION

Genetic locus: HMG20B (human) mapping to 19p13.3.

PRODUCT

BRAF35 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 BRAF35 shRNA Plasmid (h): sc-37536-SH and BRAF35 shRNA (h) Lentiviral Particles: sc-37536-V as alternate gene silencing products.

For independent verification of BRAF35 (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-37536A, sc-37536B and sc-37536C.

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

BRAF35 siRNA (h) is recommended for the inhibition of BRAF35 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

BRAF35 (F-4): sc-390813 is recommended as a control antibody for monitoring of BRAF35 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 BRAF35 gene expression knockdown using RT-PCR Primer: BRAF35 (h)-PR: sc-37536-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.