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BRCA1 shRNA (h2) Lentiviral Particles: sc-44247-V

BACKGROUND

In 1990, a breast cancer susceptibility gene, designated BRCA1, was localized to chromosome 17q. Mutations within this gene are believed to 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 13q12-q13, also confers a high incidence of breast cancer but, unlike BRCA1, does not confer a substantially elevated risk of ovarian cancer. The BRCA1 gene is expressed in numerous tissues, including breast and ovary, and encodes a predicted protein of 1,863 amino acids. This protein contains a zinc-finger domain in its amino-terminal region, but is otherwise unrelated to any previously described proteins. Like many other genes involved in familial cancer, BRCA1 appears to encode a tumor suppressor, a protein that acts as a negative regulator of tumor growth.

REFERENCES

- Hall, J.M., et al. 1990. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 250: 1684-1689.
- Narod, S.A., et al. 1991. Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 338: 82-83.
- Wooster, R., et al. 1994. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* 265: 2088-2090.
- Miki, Y., et al. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266: 66-71.
- Futreal, P.A., et al. 1994. BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266: 120-122.

CHROMOSOMAL LOCATION

Genetic locus: BRCA1 (human) mapping to 17q21.31.

PRODUCT

BRCA1 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see BRCA1 siRNA (h2): sc-44247 and BRCA1 shRNA Plasmid (h2): sc-44247-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

BRCA1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of BRCA1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

BRCA1 (G-4): sc-514640 is recommended as a control antibody for monitoring of BRCA1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor BRCA1 gene expression knockdown using RT-PCR Primer: BRCA1 (h2)-PR: sc-44247-PR (20 μ l, 479 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.