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MDC1 shRNA (h) Lentiviral Particles: sc-43917-V

BACKGROUND

MDC1, also designated nuclear factor with BRCT domains protein 1 (NFB1), participates in the early response to DNA damage. It is involved in promoting recruitment of repair proteins to the site of DNA breaks and controls damage-induced cell-cycle arrest checkpoints. The nuclear protein is a member of the BRCT (BRCA1 C-terminus) super family of nuclear proteins. It contains an N-terminus forkhead-associated (FHA) motif, two C-terminus BRCT motifs and 13 internal repetitions of a 41 amino acid sequence.

REFERENCES

1. Bradbury, J.M., et al. 2003. The complex matter of DNA double-strand break detection. *Biochem. Soc. Trans.* 31: 40-44.
2. d'Adda Di Fagagna, F., et al. 2003. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426:194-198.
3. Goldberg, M., et al. 2003. MDC1 is required for the intra-S-phase DNA damage checkpoint. *Nature* 421: 952-956.
4. Lou, Z., et al. 2003. MDC1 is coupled to activated CHK2 in mammalian DNA damage response pathways. *Nature* 421: 957-961.
5. Stewart, G.S., et al. 2003. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 421: 961-966.
6. Lou, Z., et al. 2003. Mediator of DNA damage checkpoint protein 1 regulates BRCA1 localization and phosphorylation in DNA damage checkpoint control. *J. Biol. Chem.* 278: 13599-13602.
7. Xu, X., et al. 2003. NFB1/MDC1 regulates ionizing radiation-induced focus formation by DNA checkpoint signaling and repair factors. *FASEB J.* 17: 1842-1848.

CHROMOSOMAL LOCATION

Genetic locus: MDC1 (human) mapping to 6p21.33.

PRODUCT

MDC1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl 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 MDC1 siRNA (h): sc-43917 and MDC1 shRNA Plasmid (h): sc-43917-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

MDC1 shRNA (h) Lentiviral Particles is recommended for the inhibition of MDC1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

MDC1 (C-20): sc-27737 is recommended as a control antibody for monitoring of MDC1 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MDC1 gene expression knockdown using RT-PCR Primer: MDC1 (h)-PR: sc-43917-PR (20 µl, 448 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.