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Centrin-2 shRNA (h) Lentiviral Particles: sc-43681-V

BACKGROUND

Centrin-2, an EF-hand protein, plays a critical role in normal cell division. Tissues where cilia are present, such as the retina and testis, express both Centrin-1 and -2, but Centrin-2 is also expressed in non-differentiated, non-ciliated retinal cells (retinoblastoma cells), liver, skeletal muscle, and cardiac muscle. In these tissues, centrin associates with the centrosomes, mitotic spindle poles, and basal bodies. Knockdown studies reveal a requirement for centrin in centriole duplication and organization of spindle pole morphology and the completion of cytokinesis. Additionally, Centrin-2 plays a role in nucleotide excision repair via association with xeroderma pigmentosum group C protein, suggesting possible coupling of cell division and nucleotide excision repair.

REFERENCES

1. LeDizet, M., et al. 1998. Differential regulation of centrin genes during ciliogenesis in human tracheal epithelial cells. *Am. J. Physiol.* 275: L1145-L1156.
2. Wolfrum, U., et al. 1998. Expression of centrin isoforms in the mammalian retina. *Exp. Cell. Res.* 242: 10-17.
3. Durussel, I., et al. 2000. Cation- and peptide-binding properties of human centrin 2. *FEBS Lett.* 472: 208-212.
4. Araki, M., et al. 2001. Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J. Biol. Chem.* 276: 18665-18672.
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6. Salisbury, J.L., et al. 2002. Centrin-2 is required for centriole duplication in mammalian cells. *Curr. Biol.* 12: 1287-1292.
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CHROMOSOMAL LOCATION

Genetic locus: CETN2 (human) mapping to Xq28.

PRODUCT

Centrin-2 shRNA (h) 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 Centrin-2 siRNA (h): sc-43681 and Centrin-2 shRNA Plasmid (h): sc-43681-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.

APPLICATIONS

Centrin-2 shRNA (h) Lentiviral Particles is recommended for the inhibition of Centrin-2 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

Centrin-2 (N-17)-R: sc-27793-R is recommended as a control antibody for monitoring of Centrin-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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Centrin-2 gene expression knockdown using RT-PCR Primer: Centrin-2 (h)-PR: sc-43681-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

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