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CDKN3 shRNA (m) Lentiviral Particles: sc-45278-V

BACKGROUND

Cyclin-dependent kinase inhibitor 3 (CDKN3), also designated Cdk2-associated dual specificity phosphatase, cyclin-dependent kinase interactor 1 (CDI1), CIP2, KAP or KAP1, belongs to the protein-tyrosine phosphatase family. CDKN3, a cyclin-dependent kinase inhibitor, interacts and dephosphorylates Cdk2 kinase, which prevents Cdk2 kinase activation. CDKN3 is important in cell cycle regulation. It is a dual specificity phosphatase that is active toward substrates which contain phosphotyrosine or phosphoserine residues. CDKN3 does not interact with Cdk4, but can interact with other cyclin-dependent kinases such as Cdc2, Cdk2 and Cdk3. The gene encoding for the CDKN3 protein maps to chromosome 14q22. This gene has been noted to be mutated, overexpressed or deleted in many cancers. Defects in the CDKN3 gene may be implicated in hepatocellular carcinoma (HCC).

REFERENCES

1. Gyuris, J., et al. 1993. Cdi1, a human G₁ and S phase protein phosphatase that associates with Cdk2. *Cell* 75: 791-803.
2. Hannon, G.J., et al. 1994. KAP: a dual specificity phosphatase that interacts with cyclin-dependent kinases. *Proc. Natl. Acad. Sci. USA* 91: 1731-1735.
3. Demetrick, D.J., et al. 1995. Chromosomal mapping of the genes for the human cell cycle proteins cyclin C (CCNC), cyclin E (CCNE), p21 (CDKN1) and KAP (CDKN3). *Cytogenet. Cell Genet.* 69: 190-192.
4. Yeh, C.T., et al. 2000. Aberrant transcripts of the cyclin-dependent kinase-associated protein phosphatase in hepatocellular carcinoma. *Cancer Res.* 60: 4697-4700.
5. Maak, S., et al. 2002. Rapid communication: nucleotide sequence and physical mapping of the porcine cyclin-dependent kinase inhibitor 3 (CDKN3) gene. *J. Anim. Sci.* 80: 1698-1699.

CHROMOSOMAL LOCATION

Genetic locus: Cdkn3 (mouse) mapping to 14 C1.

PRODUCT

CDKN3 shRNA (m) 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 µl frozen stock containing 1.0 x 10⁶ 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 CDKN3 siRNA (m): sc-45278 and CDKN3 shRNA Plasmid (m): sc-45278-SH as alternate gene silencing products.

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.

APPLICATIONS

CDKN3 shRNA (m) Lentiviral Particles is recommended for the inhibition of CDKN3 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

CDKN3 (S-15): sc-34165 is recommended as a control antibody for monitoring of CDKN3 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 CDKN3 gene expression knockdown using RT-PCR Primer: CDKN3 (m)-PR: sc-45278-PR (20 µl). 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.

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.