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COP1 shRNA (m) Lentiviral Particles: sc-45542-V

BACKGROUND

COP1 (Constitutive photomorphogenesis protein 1), also designated RFWD2 (ring finger and WD repeat domain 2) or RNF200 (ring finger protein 200), is an E3 ubiquitin ligase protein that mediates ubiquitination and degradation of target proteins such as c-Jun and p53. It is a component of the DCX DET1-COP1 ubiquitin ligase complex which consists of RBX1, DET1, DDB1, CUL4A and COP1. Localizing to the cytoplasm and to the nucleus, COP1 is primarily expressed in testis, placenta, heart and skeletal muscle. COP1 is a potent inhibitor of p53-dependent transcription and apoptosis but, when phosphorylated by Atm (ataxia telangiectasia mutated) in response to DNA damage, the COP1-p53 complex is disrupted and p53 is allowed to exert its pro-apoptotic properties. In ovarian and breast cancers, COP1 is overexpressed, suggesting a role for COP1 in tumorigenesis.

REFERENCES

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3. Faure, J., et al. 2004. ARF1 regulates Nef-induced CD4 degradation. *Curr. Biol.* 14: 1056-1064.
4. Feng, S., et al. 2004. *Arabidopsis* CAND1, an unmodified CUL1-interacting protein, is involved in multiple developmental pathways controlled by ubiquitin/proteasome-mediated protein Degradation. *Plant. Cell* 16: 1870-1882.
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CHROMOSOMAL LOCATION

Genetic locus: Rfwd2 (mouse) mapping to 1 H1.

PRODUCT

COP1 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 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 COP1 siRNA (m): sc-45542 and COP1 shRNA Plasmid (m): sc-45542-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

COP1 shRNA (m) Lentiviral Particles is recommended for the inhibition of COP1 expression in mouse 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

COP1 (H-300): sc-66837 is recommended as a control antibody for monitoring of COP1 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 COP1 gene expression knockdown using RT-PCR Primer: COP1 (m)-PR: sc-45542-PR (20 μ l). 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.

PROTOCOLS

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