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SANTA CRUZ BIOTECHNOLOGY, INC.

COP1 siRNA (m): sc-45542

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

1. Dornan, D., et al. 2004. COP1, the negative regulator of p53, is overexpressed in breast and ovarian adenocarcinomas. Cancer Res. 64: 7226-7230.
2. Dornan, D., et al. 2004. The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature 429: 86-92.
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.
5. McMahon, H.T., et al. 2004. COP and clathrin-coated vesicle budding: different pathways, common approaches. Curr. Opin. Cell Biol. 16: 379-391.
6. Dornan, D., et al. 2004. COP1, the negative regulator of p53, is overexpressed in breast and ovarian adenocarcinomas. Cancer Res. 64: 7226-7230.
7. Yi, C., et al. 2005. Major vault protein, in concert with constitutively photomorphogenic 1, negatively regulates c-Jun-mediated activator protein 1 transcription in mammalian cells. Cancer Res. 65: 5835-5840.
8. Qi, L., et al. 2006. TRB3 links the E3 ubiquitin ligase COP1 to lipid metabolism. Science 312: 1763-1766.

CHROMOSOMAL LOCATION

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

PRODUCT

COP1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see COP1 shRNA Plasmid (m): sc-45542-SH and COP1 shRNA (m) Lentiviral Particles: sc-45542-V as alternate gene silencing products.

For independent verification of COP1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45542A, sc-45542B and sc-45542C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

COP1 siRNA (m) is recommended for the inhibition of COP1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

COP1 (B-12): sc-166799 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

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 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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