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p53RFP siRNA (h): sc-44824

BACKGROUND

p53 is the most commonly mutated gene in human cancer identified to date. Expression of p53 leads to inhibition of cell growth by preventing progression of cells from G₁ to S phase of the cell cycle. Most importantly, p53 functions to cause arrest of cells in the G₁ phase of the cell cycle following any exposure of cells to DNA-damaging agents. The MDM2 (murine double minute-2) protein was initially identified as an oncogene in a murine transformation system. MDM2 functions to bind p53 and block p53-mediated transactivation of cotransfected reporter constructs. The MDM2 gene is amplified in a high percentage of human sarcomas that retain wildtype p53, and tumor cells that overexpress MDM2 can tolerate high levels of p53 expression. Another p53 target protein is the p53-inducible RING finger protein (p53RFP), an auto-ubiquitinated protein acting as an E3 ubiquitin ligase. p53RFP receives ubiquitin from specific E2 ubiquitin-conjugating enzymes and transfers it to substrates that promote their degradation by the proteasome. p53RFP may mediate re-entry into the cell cycle.

REFERENCES

1. Vogelstein, B. 1991. Cancer. A deadly inheritance. *Nature* 348: 681-682.
2. Hollstein, M., et al. 1991. p53 mutations in human cancers. *Science* 253: 49-53.
3. Kastan, M.B., et al. 1991. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* 51: 6304-6311.
4. Fakharzadeh, S.S., et al. 1991. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *EMBO J.* 10: 1565-1569.
5. Oliner, J.D., et al. 1993. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* 362: 857-860.
6. Chen, C.Y., et al. 1994. Interactions between p53 and MDM2 in a mammalian cell cycle checkpoint pathway. *Proc. Natl. Acad. Sci. USA* 91: 2684-2688.

CHROMOSOMAL LOCATION

Genetic locus: RNF144B (human) mapping to 6p22.3.

PRODUCT

p53RFP siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p53RFP shRNA Plasmid (h): sc-44824-SH and p53RFP shRNA (h) Lentiviral Particles: sc-44824-V as alternate gene silencing products.

For independent verification of p53RFP (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44824A, sc-44824B and sc-44824C.

PROTOCOLS

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

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

p53RFP siRNA (h) is recommended for the inhibition of p53RFP expression in human 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 μ M in 66 μ l. 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

p53RFP (U-16): sc-101247 is recommended as a control antibody for monitoring of p53RFP 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 p53RFP gene expression knockdown using RT-PCR Primer: p53RFP (h)-PR: sc-44824-PR (20 μ l). 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.