SANTA CRUZ BIOTECHNOLOGY, INC.

R1 siRNA (m): sc-37641

BACKGROUND

Ribonucleotide reductase is essential for the production and maintenance of the level of deoxyribonucleoside triphosphates (dNTP's) required for DNA synthesis. It is an enzymatic complex consisting of two nonidentical subunits, R1 and R2, which are inactive separately. R1, the larger subunit, contains allosteric regulatory sites in a human breast carcinoma cell line. R2 is the limiting factor of the catalytic activity of the ribonucleotide reductase enzymatic complex. R2 expression is strictly correlated to the S-phase of the cell cycle, whereas R1 remains constant throughout all phases of the cell cycle. Ribonucleotide reductase appears to be specifically involved in nucleotide excision repair, since both the R1 and R2 subunits are induced in response to UV light in a dose-dependent manner.

REFERENCES

1. Bjorklund, S., et al. 1990. S-phase-specific expression of mammalian ribonucleotide reductase R1 and R2 subunit mRNAs. Biochemistry 29: 5452-5458.
2. Pavloff, N., et al. 1992. Sequence analysis of the large and small subunits of human ribonucleotide reductase. DNA Seq. 2: 227-234.
3. Elledge, S.J., et al. 1992. Ribonucleotide reductase: regulation, regulation, regulation. Trends Biochem. Sci. 17: 119-123.
4. Filatov, D., et al. 1996. Induction of the mouse ribonucleotide reductase R1 and R2 genes in response to DNA damage by UV light. J. Biol. Chem. 271: 23698-23704.
5. Johansson, E., et al. 1998. Two YY-1-binding proximal elements regulate the promoter strength of the TATA-less mouse ribonucleotide reductase R1 gene. J. Biol. Chem. 273: 29816-29821.
6. Chabes, A., et al. 2000. Controlled protein degradation regulates ribonucleotide reductase activity in proliferating mammalian cells during the normal cell cycle and in response to DNA damage and replication blocks. J. Biol. Chem. 275: 17747-17753.
7. Tanaka, H., et al. 2000. A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404: 42-49.

CHROMOSOMAL LOCATION

Genetic locus: Rrm1 (mouse) mapping to 7 E3.

PRODUCT

R1 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 R1 shRNA Plasmid (m): sc-37641-SH and R1 shRNA (m) Lentiviral Particles: sc-37641-V as alternate gene silencing products.

For independent verification of R1 (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-37641A, sc-37641B and sc-37641C.

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

R1 siRNA (m) is recommended for the inhibition of R1 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

R1 (A-10): sc-377415 is recommended as a control antibody for monitoring of R1 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 R1 gene expression knockdown using RT-PCR Primer: R1 (m)-PR: sc-37641-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.