

PRIM1 siRNA (m): sc-152465

BACKGROUND

In eukaryotic cells, the replication of DNA is carried out by a variety of proteins and requires a complex chromosomal replication structure, of which POLA2 (DNA polymerase alpha) and DNA primases (PRIMs) are key components. PRIM1 (DNA primase small subunit), also known as p49, is a 420 amino acid protein that exists as a heterodimer with PRIM2A, another DNA primase. Together, PRIM1 and PRIM2A function to synthesize small RNA primers that are required for the proper activity of Okazaki fragments during replication of the DNA lagging strand. Interestingly, the gene encoding PRIM1 is coamplified with other core 12q13 amplicon genes in human osteosarcoma. In the retina of zebrafish, mutations in PRIM1 were observed to not affect cell proliferation, though neuronal apoptosis was induced. It is likely that such mutations in PRIM1 leads to activation of the ATM-Chk2-p53 apoptotic pathway.

REFERENCES

1. Adler, D.A., Tseng, B.Y., Wang, T.S. and Disteche, C.M. 1991. Physical mapping of the genes for three components of the mouse DNA replication complex: polymerase α to the X chromosome, primase p49 subunit to chromosome 10, and primase p58 subunit to chromosome 1. *Genomics* 9: 642-646.
2. Stadlbauer, F., Brueckner, A., Rehfuess, C., Eckerskorn, C., Lottspeich, F., Förster, V., Tseng, B.Y. and Nasheuer, H.P. 1994. DNA replication *in vitro* by recombinant DNA-polymerase- α -primase. *Eur. J. Biochem.* 222: 781-793.
3. Shiratori, A., Okumura, K., Nogami, M., Taguchi, H., Onozaki, T., Inoue, T., Ando, T., Shibata, T., Izumi, M. and Miyazawa, H. 1995. Assignment of the 49-kDa (PRIM1) and 58-kDa (PRIM2A and PRIM2B) subunit genes of the human DNA primase to chromosome bands 1q44 and 6p11.1-p12. *Genomics* 28: 350-353.
4. Griep, M.A. 1995. Primase structure and function. *Indian J. Biochem. Biophys.* 32: 171-178.
5. Cloutier, S., Hamel, H., Champagne, M. and Yotov, W.V. 1997. Mapping of the human DNA primase 1 (PRIM1) to chromosome 12q13. *Genomics* 43: 398-401.
6. Yotov, W.V., Hamel, H., Rivard, G.E., Champagne, M.A., Russo, P.A., Leclerc, J.M., Bernstein, M.L. and Levy, E. 1999. Amplifications of DNA primase 1 (PRIM1) in human osteosarcoma. *Genes Chromosomes Cancer* 26: 62-69.

CHROMOSOMAL LOCATION

Genetic locus: Prim1 (mouse) mapping to 10 D3.

PRODUCT

PRIM1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PRIM1 shRNA Plasmid (m): sc-152465-SH and PRIM1 shRNA (m) Lentiviral Particles: sc-152465-V as alternate gene silencing products.

For independent verification of PRIM1 (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-152465A, sc-152465B and sc-152465C.

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

PRIM1 siRNA (m) is recommended for the inhibition of PRIM1 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 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

PRIM1 (H-9): sc-390265 is recommended as a control antibody for monitoring of PRIM1 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 PRIM1 gene expression knockdown using RT-PCR Primer: PRIM1 (m)-PR: sc-152465-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.

PROTOCOLS

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