



PIG3 siRNA (h): sc-36223

BACKGROUND

The PIG (p53-induced gene) gene family encodes redox-controlling proteins that are involved in p53 tumor suppressor activity. One member of the PIG gene family, p53-inducible gene 3 (PIG3), is a p53 responsive gene that maps, in humans, to chromosome 2p and encodes a protein with significant homology to oxidoreductases. Oxidoreductases are enzymes involved in cellular responses to oxidative stress and irradiation, and they influence the involvement of PIG3 in the metabolism of reactive oxygen species. PIG3 is localized to the cytoplasm and induced in primary, non-transformed, and transformed cell cultures after exposure to genotoxic agents. The induction of PIG3 is p53 dependent and occurs with delayed kinetics as compared with other p53 downstream targets. PIG3 may act with caspase-8 as a key regulatory element in p53-dependent transcriptional deregulation by triggering the caspase cascade and mitochondrial breakdown. PIG3 is highly up-regulated by p53 and may be useful for detecting transient activation of p53.

REFERENCES

1. Polyak, K., et al. 1997. A model for p53-induced apoptosis. *Nature* 389: 300-305.
2. Flatt, P.M., et al. 2000. p53-dependent expression of PIG3 during proliferation, genotoxic stress, and reversible growth arrest. *Cancer Lett.* 156: 63-72.
3. Kovar, H., et al. 2000. Characterization of distinct consecutive phases in non-genotoxic p53-induced apoptosis of Ewing tumor cells and the rate-limiting role of caspase-8. *Oncogene* 19: 4096-4107.
4. Boulay, J.L., et al. 2000. P21 gene expression as an indicator for the activity of adenovirus-p53 gene therapy in non-small cell lung cancer patients. *Cancer Gene Ther.* 7: 1215-1219.
5. Gottifredi, V., et al. 2001. p53 accumulates but is functionally impaired when DNA synthesis is blocked. *Proc. Natl. Acad. Sci. USA* 98: 1036-1041.
6. Asher, G., et al. 2001. Regulation of p53 stability and p53-dependent apoptosis by NADH quinone oxidoreductase 1. *Proc. Natl. Acad. Sci. USA* 98: 1188-1193.

CHROMOSOMAL LOCATION

Genetic locus: TP53I3 (human) mapping to 2p23.3.

PRODUCT

PIG3 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 PIG3 shRNA Plasmid (h): sc-36223-SH and PIG3 shRNA (h) Lentiviral Particles: sc-36223-V as alternate gene silencing products.

For independent verification of PIG3 (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-36223A, sc-36223B and sc-36223C.

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

PIG3 siRNA (h) is recommended for the inhibition of PIG3 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

PIG3 (A-5): sc-166664 is recommended as a control antibody for monitoring of PIG3 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 PIG3 gene expression knockdown using RT-PCR Primer: PIG3 (h)-PR: sc-36223-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.