

PR siRNA (h2): sc-270221

BACKGROUND

The effects of progesterone are mediated by two functionally different isoforms of the progesterone receptor, PR-A and PR-B, which are transcribed from distinct, estrogen-inducible promoters within a single copy of the PR gene. The first 164 amino acids of PR-B are absent in PR-A. Progesterone-bound PR-A and PR-B have different transcription activation properties. Specifically, PR-B functions as a transcriptional activator in most cell and promoter contexts, while PR-A is transcriptionally inactive and functions as a strong ligand-dependent transdominant repressor of steroid hormone receptor transcriptional activity. An inhibitory domain (ID), which maps to the amino terminus of the receptor, exists within both PR isoforms. Interestingly, the ID is functionally active only in PR-A and is necessary for steroid hormone transrepression by PR-A, suggesting that PR-A and PR-B may have different conformations in the cell.

REFERENCES

1. Law, M.L., et al. 1987. The progesterone receptor gene maps to human chromosome band 11q13, the site of the mammary oncogene Int-2. *Proc. Natl. Acad. Sci. USA* 84: 2877-2881.
2. Kastner, P., et al. 1990. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J.* 9: 1603-1614.
3. Wen, D.X., et al. 1994. The A and B isoforms of the human progesterone receptor operate through distinct signaling pathways within target cells. *Mol. Cell. Biol.* 14: 8356-8364.
4. Beck, C.A., et al. 1996. Stoichiometry and site-specific phosphorylation of human progesterone receptor in native target cells and in the baculovirus expression system. *J. Biol. Chem.* 271: 19546-19555.
5. Zhang, Y., et al. 1997. Phosphorylation of human progesterone receptor by cyclin-dependent kinase 2 on three sites that are authentic basal phosphorylation sites *in vivo*. *Mol. Endocrinol.* 11: 823-832.
6. Giangrande, P.H., et al. 1997. Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *J. Biol. Chem.* 272: 32889-32900.

CHROMOSOMAL LOCATION

Genetic locus: PGR (human) mapping to 11q22.1.

PRODUCT

PR siRNA (h2) 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 PR shRNA Plasmid (h2): sc-270221-SH and PR shRNA (h2) Lentiviral Particles: sc-270221-V as alternate gene silencing products.

For independent verification of PR (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270221A, sc-270221B and sc-270221C.

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

PR siRNA (h2) is recommended for the inhibition of PR 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

PR (F-4): sc-166169 is recommended as a control antibody for monitoring of PR 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 PR gene expression knockdown using RT-PCR Primer: PR (h2)-PR: sc-270221-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.