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PXR shRNA (m) Lentiviral Particles: sc-44058-V

BACKGROUND

Steroid hormones function as signaling molecules by diffusing into cells and interacting with specific intracellular receptors to regulate gene expression. This superfamily of receptors includes both steroid and nonsteroid receptors. Like many nonsteroid hormone receptors, PXR (Pregnane X Receptor) binds as a heterodimer with RXR to a DNA sequence typical of a nonsteroid hormone receptor; however, PXR is activated by several steroids, such as naturally occurring pregnanes and synthetic glucocorticoids and anti-glucocorticoids. PXR exists as two alternatively spliced isoforms, PXR.1 and PXR.2. PXR is thought to define a novel steroid hormone signaling pathway that may account for some of the effects of synthetic glucocorticoids and antiglucocorticoids that are not mediated through the classical glucocorticoid receptor signaling pathway.

REFERENCES

1. Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* 240: 889-895.
2. Mangelsdorf, D.J., et al. 1995. The RXR heterodimers and orphan receptors. *Cell* 83: 841-850.
3. Huss, J.M., et al. 1996. Dexamethasone responsiveness of a major glucocorticoid-inducible CYP3A gene is mediated by elements unrelated to a glucocorticoid receptor binding motif. *Proc. Natl. Acad. Sci. USA* 93: 4666-4670.

CHROMOSOMAL LOCATION

Genetic locus: Nr1i2 (mouse) mapping to 16 B3.

PRODUCT

PXR shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PXR siRNA (m): sc-44058 and PXR shRNA Plasmid (m): sc-44058-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

PXR shRNA (m) Lentiviral Particles is recommended for the inhibition of PXR expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PXR (H-160): sc-25381 is recommended as a control antibody for monitoring of PXR 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PXR gene expression knockdown using RT-PCR Primer: PXR (m)-PR: sc-44058-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

PROTOCOLS

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