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CAR1/2 shRNA (m) Lentiviral Particles: sc-43663-V

BACKGROUND

The CAR (constitutively acting receptor) proteins, CAR1 and CAR2, are mouse nuclear hormone receptors. CAR1 and CAR2, along with their human homolog, MB67, are in highest expression in the liver and belong to a group of receptors known as orphan receptors due to their lack of a known ligand. Unlike conventional hormone receptors which activate transcription upon binding with steroids, retinoids and thyroid hormones, the CAR and MB67 orphan receptors are transcriptionally active in the absence of exogenous hormone. The CAR and MB67 orphan receptors bind to DNA in the form of a heterodimer with the retinoic-X receptor and activate gene transcription in a constitutive manner.

REFERENCES

1. Davies, P., et al. 1988. The structure and function of steroid receptors. *Sci. Prog.* 72: 563-578.
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3. Mangelsdorf, D.J., et al. 1995. The RXR heterodimers and orphan receptors. *Cell* 83: 841-850.
4. Choi, H.S., et al. 1997. Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J. Biol. Chem.* 272: 23565-23571.
5. Forman, B.M., et al. 1998. Androstane metabolites bind to and deactivate the nuclear receptor CAR- β . *Nature* 395: 612-615.
6. Frank, C., et al. 2003. Characterization of DNA complexes formed by the nuclear receptor constitutive androstane receptor. *J. Biol. Chem.* 278: 43299-43310.
7. Burk, O., et al. 2004. The induction of cytochrome P450 3A5 (CYP3A5) in the human liver and intestine is mediated by the xenobiotic sensors pregnane X receptor (PXR) and constitutively activated receptor (CAR). *J. Biol. Chem.* 279: 38379-38385.

CHROMOSOMAL LOCATION

Genetic locus: Nr1i3 (mouse) mapping to 1 H3.

PRODUCT

CAR1/2 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 CAR1/2 siRNA (m): sc-43663 and CAR1/2 shRNA Plasmid (m): sc-43663-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.

APPLICATIONS

CAR1/2 shRNA (m) Lentiviral Particles is recommended for the inhibition of CAR 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

CAR1/2 (M-127): sc-13065 is recommended as a control antibody for monitoring of CAR 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 CAR gene expression knockdown using RT-PCR Primer: CAR1/2 (m)-PR: sc-43663-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

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.

PROTOCOLS

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