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Cox-2 shRNA (h2) Lentiviral Particles: sc-44256-V

BACKGROUND

Prostaglandins are a diverse group of autocrine and paracrine hormones that mediate many cellular and physiologic processes. Prostaglandin H2 (PGH2) is an intermediate molecule in formation of the prostaglandins. Cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2) are prostaglandin synthases that catalyze the formation of PGH2 from arachidonic acid (AA). Cox-1 and Cox-2 are isozymes of prostaglandin-endoperoxidase synthase (PTGS). Cox-1 is constitutively expressed in most tissues and is thought to serve in general "housekeeping" functions. Cox-2 is efficiently induced in migratory cells responding to pro-inflammatory stimuli and is considered to be an important mediator of inflammation. Both enzymes are targets for the nonsteroidal therapeutic anti-inflammatory drugs, NSAIDs.

REFERENCES

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- Langenbach, R., et al. 1995. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83: 483-492.
- Tsuji, M., et al. 1995. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxidase synthase 2. *Cell* 83: 493-501.
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CHROMOSOMAL LOCATION

Genetic locus: PTGS2 (human) mapping to 1q31.1.

PRODUCT

Cox-2 shRNA (h2) 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 Cox-2 siRNA (h2): sc-44256 and Cox-2 shRNA Plasmid (h2): sc-44256-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

Cox-2 shRNA (h2) Lentiviral Particles is recommended for the inhibition of Cox-2 expression in human 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

Cox-2 (D-12): sc-166475 is recommended as a control antibody for monitoring of Cox-2 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Cox-2 gene expression knockdown using RT-PCR Primer: Cox-2 (h2)-PR: sc-44256-PR (20 μ l, 476 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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