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EP2 shRNA (r) Lentiviral Particles: sc-45910-V

BACKGROUND

Prostaglandin E2, a member of the autacoid family of lipid mediators, is a major renal cyclooxygenase product of arachidonic acid metabolism. Prostaglandin E2 binds to four G protein-coupled E-prostanoid receptors, designated EP1, EP2, EP3 and EP4. The expression and function of the prostaglandin E2 receptors have been highly characterized in kidney. EP1, which is predominantly expressed in the collecting duct, couples to G_q proteins to inhibit sodium absorption and increase in intracellular calcium, which act as second messengers. EP2 is coupled to G_s proteins, which stimulate adenylyl cyclase. EP2 has the lowest expression in kidney, but EP2 knockout mice exhibit salt-sensitive hypertension, which suggests a role for EP2 in salt excretion. EP3 is expressed in renal vessels, thick ascending limb and collecting duct. EP3 has at least six alternative splice variants that couple to G_i proteins to inhibit cAMP, which subsequently inhibit sodium and water transport. In uterus, EP3 induces the contraction of uterine smooth muscles. EP4 is expressed in glomerulus and collecting duct. It couples to G_s proteins, which stimulate adenylyl cyclase and regulate glomerular tone and renal renin release.

REFERENCES

1. Breyer, M.D., et al. 1998. Regulation of renal function by prostaglandin E receptors. *Kidney Int. Suppl.* 67: S88-S94.
2. Ichikawa, A. 1998. Molecular biology of prostaglandin E receptors—expression of multi-function by PGE receptor subtypes and isoforms. *Nippon Rinsho* 56: 1813-1818.
3. Kotani, M., et al. 2000. Multiple signal transduction pathways through two prostaglandin E receptor EP3 subtype isoforms expressed in human uterus. *J. Clin. Endocrinol. Metab.* 85: 4315-4322.
4. Muro, S., et al. 2000. Expression of prostaglandin E receptor EP4 subtype in rat adrenal zona glomerulosa: involvement in aldosterone release. *Endocr. J.* 47: 429-436.
5. Thiernemann, C., et al. 2000. Selective activation of E-type prostanoid 3 receptors reduces myocardial infarct size. A novel insight into the cardio-protective effects of prostaglandins. *Pharmacol. Ther.* 87: 61-67.

CHROMOSOMAL LOCATION

Genetic locus: Ptger2 (rat) mapping to 15p14.

PRODUCT

EP2 shRNA (r) 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 µl frozen stock containing 1.0 x 10⁶ 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 EP2 siRNA (r): sc-45910 and EP2 shRNA Plasmid (r): sc-45910-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

EP2 shRNA (r) Lentiviral Particles is recommended for the inhibition of EP2 expression in rat cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

EP2 (H-75): sc-20675 is recommended as a control antibody for monitoring of EP2 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 EP2 gene expression knockdown using RT-PCR Primer: EP2 (r)-PR: sc-45910-PR (20 µl). 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.

PROTOCOLS

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