SANTA CRUZ BIOTECHNOLOGY, INC.

EP4 siRNA (h): sc-40173

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.

CHROMOSOMAL LOCATION

Genetic locus: PTGER4 (human) mapping to 5p13.1.

PRODUCT

EP4 siRNA (h) is a target-specific 19-25 nt siRNA designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EP4 shRNA Plasmid (h): sc-40173-SH and EP4 shRNA (h) Lentiviral Particles: sc-40173-V as alternate gene silencing products.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

EP4 siRNA (h) is recommended for the inhibition of EP4 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 µM in 66 µl. 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

EP4 (C-4): sc-55596 is recommended as a control antibody for monitoring of EP4 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 EP4 gene expression knockdown using RT-PCR Primer: EP4 (h)-PR: sc-40173-PR (20 µl, 585 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Vo, B.T., et al. 2013. TGF-β effects on prostate cancer cell migration and invasion are mediated by PGE2 through activation of PI3K/Akt/mTOR pathway. Endocrinology 154: 1768-1779.
2. Fan, Y., et al. 2015. Prostaglandin E2 stimulates normal bronchial epithelial cell growth through induction of c-Jun and PDK1, a kinase implicated in oncogenesis. Respir. Res. 16: 149.
3. Zhong, X., et al. 2015. Novel link between prostaglandin E2 (PGE2) and cholinergic signaling in lung cancer: the role of c-Jun in PGE2-induced α7 nicotinic acetylcholine receptor expression and tumor cell proliferation. Thorac. Cancer 6: 488-500.
4. Fan, Y., et al. 2017. Nicotine induces EP4 receptor expression in lung carcinoma cells by acting on AP-2α: the intersection between cholinergic and prostanoid signaling. Oncotarget 8: 75854-75863.
5. Liao, C.W., et al. 2019. Interleukin-6 plays a critical role in aldosterone-induced macrophage recruitment and infiltration in the myocardium. Biochim. Biophys. Acta Mol. Basis Dis. 1866: 165627.

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.