

## EP3 siRNA (m): sc-35315

### 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<sub>q</sub> proteins to inhibit sodium absorption and increase in intracellular calcium, which act as second messengers. EP2 is coupled to G<sub>s</sub> 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<sub>i</sub> 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<sub>s</sub> 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. Thiernemann, C. and Zacharowski, K. 2000. Selective activation of E-type prostanoid<sub>3</sub>-receptors reduces myocardial infarct size. A novel insight into the cardioprotective effects of prostaglandins. *Pharmacol. Ther.* 87: 61-67.
4. Breyer, M.D. and Breyer, R.M. 2000. Prostaglandin E receptors and the kidney. *Am. J. Physiol. Renal Physiol.* 279: F12-F23.
5. 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.
6. 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.

### CHROMOSOMAL LOCATION

Genetic locus: Ptger3 (mouse) mapping to 3 H4.

### PRODUCT

EP3 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EP3 shRNA Plasmid (m): sc-35315-SH and EP3 shRNA (m) Lentiviral Particles: sc-35315-V as alternate gene silencing products.

For independent verification of EP3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35315A, sc-35315B and sc-35315C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

EP3 siRNA (m) is recommended for the inhibition of EP3 expression in mouse 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  $\mu$ M in 66  $\mu$ l. 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

EP3 (5F5): sc-57105 is recommended as a control antibody for monitoring of EP3 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EP3 gene expression knockdown using RT-PCR Primer: EP3 (m)-PR: sc-35315-PR (20  $\mu$ l, 560 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### RESEARCH USE

For research use only, not for use in diagnostic procedures.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.