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Epac2 siRNA (h): sc-41702

BACKGROUND

3',5' cyclic adenosine monophosphate (cAMP)-regulated guanine nucleotide exchange factors Epac1 (cAMP-GEFI) and Epac2 (cAMP-GEFII) activate the Ras family GTPases Rap 1 and Rap 2 by promoting GTP binding in a cAMP-dependent manner. Eukaryotic cAMP is a second messenger that induces physiological responses such as gene expression, growth, differentiation, secretion and neurotransmission. Human EPAC2 contains at least 31 exons and maps to chromosome 2q31.1. The 4.4-kb Epac2 transcript is prominent in brain and adrenal gland. Within the brain, expression is strong in cortex, occipital pole, frontal lobe, temporal lobe, amygdala, putamen, hippocampus and cerebellum.

REFERENCES

1. Kawasaki, H., et al. 1998. A family of cAMP-binding proteins that directly activate Rap 1. *Science* 282: 2275-2279.
2. de Rooij, J., et al. 2000. Mechanism of regulation of the Epac family of cAMP-dependent RAPGEFs. *J. Biol. Chem.* 275: 20829-20836.
3. Ueno, H., et al. 2001. Characterization of the gene EPAC2: structure, chromosomal localization, tissue expression, and identification of the liver-specific isoform. *Genomics* 78: 91-98.
4. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 606058. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Fujimoto, K., et al. 2002. Piccolo, a Ca²⁺ sensor in pancreatic β-cells. Involvement of cAMP-GEFII.Rim2. Piccolo complex in cAMP-dependent exocytosis. *J. Biol. Chem.* 277: 50497-50502.

CHROMOSOMAL LOCATION

Genetic locus: RAPGEF4 (human) mapping to 2q31.1.

PRODUCT

Epac2 siRNA (h) 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Epac2 shRNA Plasmid (h): sc-41702-SH and Epac2 shRNA (h) Lentiviral Particles: sc-41702-V as alternate gene silencing products.

For independent verification of Epac2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41702A, sc-41702B and sc-41702C.

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

Epac2 siRNA (h) is recommended for the inhibition of Epac2 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

Epac2 (A-7): sc-28326 is recommended as a control antibody for monitoring of Epac2 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 Epac2 gene expression knockdown using RT-PCR Primer: Epac2 (h)-PR: sc-41702-PR (20 μl, 440 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Haag, S., et al. 2008. Role of Epac1 in mediating anti-proliferative effects of prostanoid EP₂ receptors and cAMP in human lung fibroblasts. *Naunyn Schmiedebergs Arch. Pharmacol.* 378: 617-630.
2. Yoshie, M., et al. 2010. Possible role of the exchange protein directly activated by cyclic AMP (Epac) in the cyclic AMP-dependent functional differentiation and syncytialization of human placental BeWo cells. *Hum. Reprod.* 25: 2229-2238.

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.