



Epac siRNA (r): sc-270246

BACKGROUND

3',5' cyclic adenosine monophosphate (cAMP)-regulated guanine nucleotide exchange factors Epac (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. The human Epac gene maps to chromosome 12q13.11 with transcript being abundant in the kidney and heart. *In situ* hybridization indicates expression of Epac in adult rat brain and selective expression in neonatal brain, including septum and thalamus.

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. 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.
5. Online Mendelian Inheritance in Man, OMIM™. 2005. Johns Hopkins University, Baltimore, MD. MIM Number: 606057. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. LocusLink Report (LocusID: 10411). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: Rapgef3 (rat) mapping to 7q36.

PRODUCT

Epac siRNA (r) is a pool of 2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Epac shRNA Plasmid (r): sc-270246-SH and Epac shRNA (r) Lentiviral Particles: sc-270246-V as alternate gene silencing products.

For independent verification of Epac (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270246A and sc-270246B.

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

APPLICATIONS

Epac siRNA (r) is recommended for the inhibition of Epac expression in rat 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 μ M in 66 μ 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

Epac (A-5): sc-28366 is recommended as a control antibody for monitoring of Epac 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Epac gene expression knockdown using RT-PCR Primer: Epac (r)-PR: sc-270246-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Heldsinger, A., et al. 2014. Ghrelin induces leptin resistance by activation of suppressor of cytokine signaling 3 expression in male rats: implications in satiety regulation. *Endocrinology* 155: 3956-3969.
2. Michael, E.S., et al. 2019. Trace amine-associated receptor 1 (TAAR1) promotes anti-diabetic signaling in Insulin-secreting cells. *J. Biol. Chem.* 294: 4401-4411.

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.