

PACAP siRNA (r): sc-270540

BACKGROUND

The vasoactive intestinal peptide (VIP) and the pituitary adenylate cyclase-activating polypeptide (PACAP) belong to a superfamily of peptide hormones that include glucagon, secretin and growth hormone releasing hormone. The effects of VIP and PACAP are mediated by three G protein-coupled receptors, VPAC1, VPAC2 and the PACAP receptor (also designated PAC1-R). The VPAC receptors have equal affinities for VIP and PACAP, which stimulate the activation of adenylyl cyclase. Both VPAC1 and VPAC2 are abundantly expressed in brain and T cells, where they modulate neuronal differentiation and T cell activation, respectively. The PACAP receptor is a seven transmembrane protein that produces at least eight isoforms by alternative splicing. Each isoform is associated with a specific signaling pathway and a specific expression pattern. The PACAP receptor, which is thought to play an integral role in brain development, preferentially binds PACAP in order to stimulate a cAMP-protein kinase A signaling pathway.

REFERENCES

1. Shen, S., et al. 2000. Overexpression of the human VPAC2 receptor in the suprachiasmatic nucleus alters the circadian phenotype of mice. *Proc. Natl. Acad. Sci. USA* 97: 11575-11580.
2. Shioda, S. 2000. Pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors in the brain. *Kaibogaku Zasshi* 75: 487-507.
3. Bajo, A.M., et al. 2000. Expression of vasoactive intestinal peptide (VIP) receptors in human uterus. *Peptides* 21: 1383-1388.
4. Karacay, B., et al. 2000. Regulation of vasoactive intestinal peptide receptor expression in developing nervous systems. *Ann. N.Y. Acad. Sci.* 921: 165-174.
5. Vaudry, D., et al. 2000. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev.* 52: 269-324.
6. Henning, R.J., et al. 2001. Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc. Res.* 49: 27-37.
7. Lara-Marquez, M., et al. 2001. Selective gene expression and activation-dependent regulation of vasoactive intestinal peptide receptor type 1 and type 2 in human T cells. *J. Immunol.* 166: 2522-2530.

CHROMOSOMAL LOCATION

Genetic locus: *Adcyap1* (rat) mapping to 9q38.

PRODUCT

PACAP 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 PACAP shRNA Plasmid (r): sc-270540-SH and PACAP shRNA (r) Lentiviral Particles: sc-270540-V as alternate gene silencing products.

For independent verification of PACAP (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-270540A and sc-270540B.

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

PACAP siRNA (r) is recommended for the inhibition of PACAP 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

PACAP (F-2): sc-166180 is recommended as a control antibody for monitoring of PACAP 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 PACAP gene expression knockdown using RT-PCR Primer: PACAP (r)-PR: sc-270540-PR (20 μ l). 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 for detailed protocols and support products.