

# ASIC4 siRNA (h): sc-42411

## BACKGROUND

Degenerin/epithelial sodium channel (DEG/ENaC) superfamily members are amiloride-sensitive sodium channels that contain intracellular N- and C-termini, two hydrophobic transmembrane regions and a cysteine-containing extracellular loop. Acid sensing ion channel ASIC1, also designated ACCN2, BNAC2 and ASIC1  $\alpha$ , is present in brain as a 4.3-kb transcript with localization to rat dorsal root ganglia. *In situ* hybridization of rat brain suggests that ASIC1 is most abundant in the main olfactory bulb, cerebral cortex, hippocampal formation, habenula, basolateral amygdaloid nuclei and cerebellum. ASIC1 and H<sup>+</sup>-gated currents may contribute to the development of fear and anxiety. ASIC2, also designated ACCN1 (amiloride-sensitive cation channel 1, neuronal), mammalian degenerin, BNAC1 (MDEG) and brain Na<sup>+</sup> channel 1 mediate the normal detection of light touch. ASIC2 mRNA is abundant in brain, specifically in neurons. ASIC2 is expressed as 2.7 and 3.7 kb transcripts in brain and spinal cord tissues. ASIC3, also designated SLNAC1 and TNaC1, mediates detection of lasting pH changes and is involved in modulating moderate- to high-intensity pain sensation. ASIC4, also designated ACCN4 and BNAC4, is abundant in pituitary gland and is also present in the inner ear.

## REFERENCES

1. Garcia-Anoveros, J., et al. 1997. BNAC1 and BNAC2 constitute a new family of human neuronal sodium channels related to degenerins and epithelial sodium channels. *Proc. Natl. Acad. Sci. USA* 94: 1459-1464.
2. Waldmann, R., et al. 1997. A proton-gated cation channel involved in acid-sensing. *Nature* 386: 173-177.
3. Price, M.P., et al. 2000. The mammalian sodium channel BNC1 is required for normal touch sensation. *Nature* 407: 1007-1011.
4. Grunder, S., et al. 2001. Acid-sensing ion channel (ASIC) 4 gene: physical mapping, genomic organisation, and evaluation as a candidate for paroxysmal dystonia. *Eur. J. Hum. Genet.* 9: 672-676.
5. Chen, C.C., et al. 2002. A role for ASIC3 in the modulation of high-intensity pain stimuli. *Proc. Natl. Acad. Sci. USA* 99: 8992-8997.
6. Wemmie, J.A., et al. 2004. Overexpression of acid-sensing ion channel 1a in transgenic mice increases acquired fear-related behavior. *Proc. Natl. Acad. Sci. USA* 101: 3621-3626.

## CHROMOSOMAL LOCATION

Genetic locus: ACCN4 (human) mapping to 2q35.

## PRODUCT

ASIC4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ASIC4 shRNA Plasmid (h): sc-42411-SH and ASIC4 shRNA (h) Lentiviral Particles: sc-42411-V as alternate gene silencing products.

For independent verification of ASIC4 (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-42411A, sc-42411B and sc-42411C.

## 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

ASIC4 siRNA (h) is recommended for the inhibition of ASIC4 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  $\mu$ 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

ASIC4 (E-8): sc-514818 is recommended as a control antibody for monitoring of ASIC4 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 ASIC4 gene expression knockdown using RT-PCR Primer: ASIC4 (h)-PR: sc-42411-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.