

## Product Information

### Anti-Hyperpolarization-Activated Cation Channel HCN2, antibody developed in rabbit affinity isolated antibody

Catalog Number **H2769**

#### Product Description

Anti-Hyperpolarization-Activated Cation Channel HCN2 is developed in rabbit using a highly purified peptide (C)EEAGPAGEPRGSQAS (HCN2 147-161), corresponding to amino acid residues 147-161 of human HCN2<sup>1,2</sup> with an additional N-terminal cysteine as an immunogen. The antibody was affinity isolated on immobilized HCN2 147-161.

Anti-Hyperpolarization-Activated Cation Channel HCN2 recognizes HCN2 protein from rat brain by immunoblotting.

Hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels are involved in pacemaker activity of cardiac sinoatrial node myocytes and central neurons. Cardiac pacemaking determines the heart rate and rhythm, and is generated by the slow membrane-depolarization period that occurs between action potentials.<sup>3</sup> A hyperpolarization-activated current,  $I_h$  (or  $I_f$ ), is an important part of the ionic conductance responsible for cardiac pacemaker depolarization.  $I_h$ , which is carried by both  $\text{Na}^+$  and  $\text{K}^+$ , is activated by membrane hyperpolarization. Rising cAMP levels result in increased inward current at a fixed negative membrane potential. This mechanism is responsible for heart rate acceleration in response to sympathetic stimulation.<sup>4</sup> cAMP binds directly to the channel to regulate current.<sup>5</sup> An  $I_h$  current exists in a variety of neuronal cells as well. A major function of the current in the brain is to regulate neuronal pacemaking, the rate of rhythmic oscillations of single neurons and neuronal networks.<sup>6,7</sup>

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels belong to the superfamily of voltage-gated cation channels.<sup>8</sup> Their features include: six transmembrane helices (S1-S6) and an ion-conducting P region between the fifth and sixth segment. In addition, HCN channels contain a cyclic nucleotide-binding domain (CNBD) in this C-terminus. This region allows the channel to be modulated by direct interaction with cAMP or cGMP.<sup>1</sup>

HCN2, in particular, is expressed in mouse and human brain and heart.<sup>1</sup> Among the four HCN channels identified, HCN2 is the only channel expressed in both places. HCN2 mRNA is highly expressed and nearly ubiquitous in the brain. The most prominent signals are seen in the hippocampus, thalamus, and brain stem. The activation kinetics of HCN2 suggest its activity may correlate with the fast component of the cardiac  $I_h$ .<sup>2</sup>

Although the functional role of the HCN channels is becoming clear, the physiological role is less straightforward. There are several disorders of pacemaking, such as congenital sinus node dysfunction. Future work will investigate the possibility that these diseases are linked to mutations of the HCN genes.

#### Reagents

Anti-Hyperpolarization-Activated Cation Channel HCN2 is supplied lyophilized at 0.3 mg/ml from Phosphate Buffered Saline, pH 7.4, containing 1% Bovine Serum Albumin, and 0.05% Sodium Azide.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

Reconstitute the lyophilized vial with 0.05 ml or 0.2 ml deionized water, depending on the package size purchased. Antibody dilutions should be made in buffer containing 1–3% bovine serum albumin.

#### Storage/Stability

Prior to reconstitution, store at  $-20^\circ\text{C}$ . After reconstitution, the stock antibody solution may be stored at  $4^\circ\text{C}$  for up to 2 weeks. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

**Product Profile**

The recommended working dilution is 1:200-1:300 (1.0-1.5 µg/ml) for immunoblotting using HRP-goat anti-rabbit and detection by ECL.

Note: In order to obtain the best results using various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

**References**

1. Ludwig, A. et al., *Nature*, **393**, 587 (1998).
2. Ludwig, A. et al., *EMBO J.*, **18**, 2323 (1999).
3. DiFrancesco, D., *Annu. Rev. Physiol.*, **55**, 455 (1993).
4. Brown, H.F. et al., *Nature*, **280**, 235 (1979).
5. DiFrancesco, D., and Mangoni, M., *J. Physiol.*, **474**, 473 (1994).
6. Pape, H.C., *Ann. Rev. Physiol.*, **58**, 299 (1996).
7. Luthi, A., and McCormick, D.A., *Neuron*, **21**, 9 (1998).
8. Zagotta, W.N. and Siegelbaum, S.A., *Annu. Rev. Neurosci.*, **19**, 235 (1996).

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