

Product Specification Sheet

Hyperpolarization-activated Cyclic Nucleotide-gated channel Pore (HCN1-pore) Antibodies

Cat. # HCNP15-S	Rabbit Anti-Human HCN1-pore antiserum	SIZE: 100 ul
Cat. # HCNP15-A	Rabbit Anti-Human HCN1-Pore IgG (Aff pure)	SIZE: 100 ug
Cat. # HCNP15-P	Human HCN1-pore Control/blocking peptide	SIZE: 100 ug

Cardiac pacemaking is produced by the slow diastolic depolarization phase of the action potential. The hyperpolarization-activated cation current (termed I_i , I_h , or I_q) plays a key role in the in the initiation and modulation of cardiac and neuronal pacemaker depolarization. The I_i channel has been designated as "pacemaker" channel because it reveals unique features that are believed to be a prerequisite for pacemaker activity. Recently, the **hyperpolarization-activated cyclic nucleotide-gated families of ion channel proteins (HCN1-4)** have been identified as the "pacemaker" channel. The amino acid sequences of HCNs predict a structure similar to that of voltage-gated (Kv) channels and cyclic nucleotide-gated (CNG) channels. HCNs proteins (HCN1-4) are characterized by six transmembrane domains (S1-S6), including a including a positively charged voltage-sensing S4 segment and an **ion-conducting pore** between S5 and S6. In the C terminus the HCNs carry a cyclic nucleotide-binding domain (CNBD), a motif found in several cyclic nucleotide-binding proteins. The core region of HCNs channels (S1 to the C terminus of the CNBD) is highly conserved, whereas the cytoplasmic N and C-termini, vary considerably in their length and share only weak sequence homology.

HCN channels pass both Na^+ and K^+ with relative permeability ratio of ~0.15 to 0.25. The primary sequence of HCN pore region is clearly related to that of highly selective K^+ channels. In contrast to nonselective CNG or Na^+ -channels, both K^+ and HCN channels contain a GYG motif in the pore. By affecting the spatial coordination of the GYG sequence in the tetrameric channel complex, other channel domains could control the structural rigidity of the pore and thereby determine whether only K^+ or also other monovalent are allowed to permeate.

Source of Antigen and Antibodies

Antigen	23-aa (SYA LFK AMS HML CIG YGA QAP VS, human HCN1 pore 225-247-aa; designated HCNP15-P or control peptide /blocking peptide) conjugated to KLH; ~ pore region
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#HCNP15-S) and IgG, purified over antigen-agarose (Cat # HCNP15-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as -ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Affinity pure IgG

100 ug/100ul solution lyophilized powder
Supplied in **Buffer:** PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide

100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at -20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: not tested. We recommend the use of affinity pure antibody at 2-20 ug/ml.

Specificity & Cross-reactivity

The pore region of HCN1 (HCNP15-P) is very conserved among various HCNs. **HCN1:** mouse, rat, human, rabbit=100%; **HCN2:** mouse, rat, human (86%); **HCN3:** mouse (95%), rat (90%); **HCN4:** rat, human (95%). The HCNP15-P sequence is also conserved in insect (Helothis virescens, 78%), Drosophila (78%) and Sea urchin (S. purpuratus, 69%) HCNs. Therefore, antibodies to HCNP15-P should react with most HCNs and related sequences. Antibody reactivity in various species is not known. The HCNP15-P Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at the web site).

General References: (1) Santoro B et al (1997) PNAS 94, 14815-14820; Santoro B et al (1998) Cell 93, 717-729; Steven DR et al (2001) Nature 413, 631-635; Waigner BJ et al (2001) Nature 411, 825-810; Ludwig A et al (1998) Nature 393, 587-591; Biel M et al (2002) Trend Cardiovasc. Med. 12, 206-213 (review)

**This product is for In vitro research use only.*

Related materials available from ADI

HCNP15-S-A-P 71214A

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