abcam

Product datasheet

Anti-HCN4 antibody [SHG 1E5] ab32675

21 References 4 Images

Overview

Product name	Anti-HCN4 antibody [SHG 1E5]
Description	Rat monoclonal [SHG 1E5] to HCN4
Host species	Rat
Tested applications	**Suitable for:** WB, ICC/IF, IHC-Fr, IHC-FoFr, Flow Cyt, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chimpanzee
Immunogen	Synthetic peptide corresponding to Human HCN4 aa 1048-1085. Sequence: SHGSLLLPPASSPPPPQVPQRRGTPPLTPGRLTQDLK L Database link: Q9Y3Q4 Run BLAST with Run BLAST with
Positive control	IHC-P: Normal and cancer biopsies of human heart tissue. Normal and cancer biopsies of human tonsil tissue. WB: Rat eye lysate. Flow Cytometry: PC-12 cells.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: Ascites
Purity	Ascites
Clonality	Monoclonal
Clone number	SHG 1E5
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab32675** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/5000. Detects a band of approximately 132 kDa (predicted molecular weight: 130 kDa).
ICC/IF		Use at an assay dependent concentration. PubMed: 19775764
IHC-Fr		1/1000.
IHC-FoFr		Use at an assay dependent concentration. PubMed: 29403069
Flow Cyt		1/50. ab18407 - Rat monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P		1/10 - 1/100.

Target

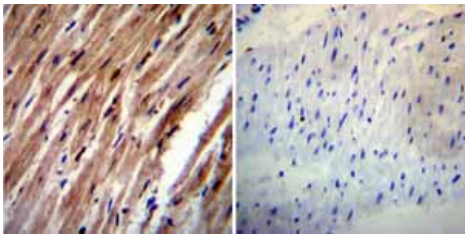
Relevance

HCN4 is a member of the family of hyperpolarization activated and cyclic nucleotide gated (HCN) channels. HCN currents have been linked to pacemaker activity in the heart and brain, resting potential control, as well as neuronal plasticity. It has been shown that HCN4 channels function as receptors for sour taste, and are associated with pacemaker potential generation in the sinoatrial node.

Cellular localization

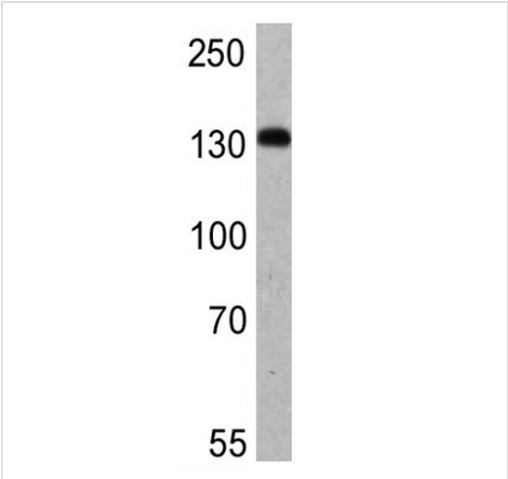
Membrane; multi pass membrane protein.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HCN4 antibody [SHG 1E5] (ab32675)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human heart tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10 mM sodium citrate (pH 6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a rat monoclonal antibody recognizing HCN4 ab32675 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Western blot - Anti-HCN4 antibody [SHG 1E5] (ab32675)

Anti-HCN4 antibody [SHG 1E5] (ab32675) at 1/200 dilution + Rat eye lysate at 25 µg

Secondary

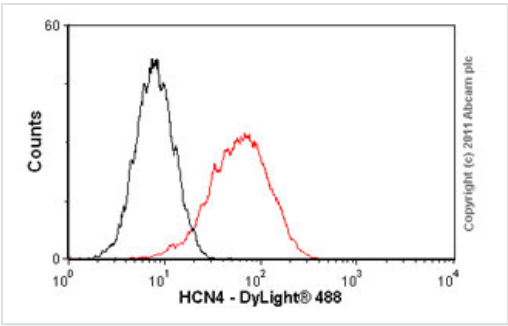
HRP Conjugate

Developed using the ECL technique.

Predicted band size: 130 kDa

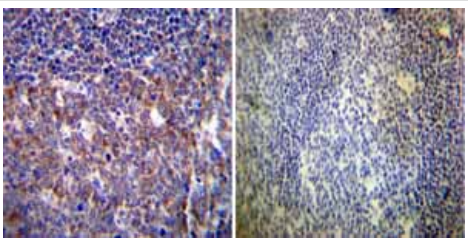
Observed band size: 132 kDa

why is the actual band size different from the predicted?



Flow Cytometry - Anti-HCN4 antibody [SHG 1E5] (ab32675)

Overlay histogram showing PC-12 (Rat adrenal gland pheochromocytoma cell line) cells stained with ab32675 (red line). The cells were fixed with methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32675, 1/50 dilution) for 30 minutes at 22°C. The secondary antibody used was DyLight® 488 goat anti-rat IgG (H+L) (ab98386) at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rat IgG1 [RTK2071] (ab18412, 2 µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in PC-12 cells fixed with 4% paraformaldehyde (10 minutes)/permeabilized with 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HCN4 antibody [SHG 1E5] (ab32675)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human tonsil tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10 mM sodium citrate (pH 6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a rat monoclonal antibody recognizing HCN4 ab32675 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-

conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

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