SOPHION BIOSCIENCE | Discover more

# Application Report

## Electrophysiological characterization of axoCells™ hiPSC-derived ventricular cardiomyocytes using physiological solutions on QPatch

Pharmacological and biophysical evaluation of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), including cardiac voltage-gated ion channels ($Na_V1.5$ and $Ca_V1.2$) and paced action potentials


## Summary

Adapting our specialized hiPSC-CM dissociation protocols and automated patch clamp (APC) assays we characterized axoCells™ hiPSC-CMs in physiological solutions using QPatch obtaining:

- Up to 40% whole-cell (WC) success rate in physiological solutions

Pharmacological and biophysical evaluation identified:

- $Na_V1.5$ currents in 80% of the hiPSC-CMs
- $Ca_V1.2$ currents in 60% of the hiPSC-CMs
- Paced action potentials in 60% of the hiPSC-CMs


## Introduction

Cardiovascular disease remains the leading global cause of death, and the evaluation of potential cardiotoxicity continues to be a major challenge and expense in early drug development. The human-induced pluripotent stem-cell (hiPSC) technology was developed in 2007[1,2] and hiPSC-derived cardiomyocytes (hiPSC-CMs) have since then been evaluated as a promising model system for cardiac drug screening, disease modelling and cardiotoxicity testing[3,4].

Automated patch clamp (APC) systems enable high-throughput, precise electrophysiological measurements of cardiac ion channels across large hiPSC-CM populations, accelerating screening workflows of these key therapeutic and safety targets.

This study employs the Sophion QPatch APC platform to biophysically and pharmacologically evaluate cardiac ion channels and paced action potentials in axoCells™ ventricular hiPSC-CMs, to highlight the potential of this technology in cardiac safety and drug discovery.

## Results and discussion

### Optimization of axoCells™ hiPSC-CM dissociation and APC assays

By adapting existing operating procedures, we optimized the dissociation and whole-cell protocol for axoCells™ hiPSC-CMs, resulting in up to 40% whole-cell success rate in physiological solutions on QPatch (Fig. 1A and B). The axoCells™ hiPSC-CMs were recorded at day *in vitro* (DIV) 14. Sealing resistances were good, with a mean membrane resistance ($R_{mem}$) above 1.5 GΩ (Fig. 1C) and the cell capacitances ($C_{slow}$) were heterogeneous, ranging from 5 pF to 45 pF, corresponding to a large variation in hiPSC-CM sizes (Fig. 1D). As the hiPSC CMs mature, their size increases, so it is expected that the proportion of $C_{slow}$ values above 20 pF will increase if culture time is extended.

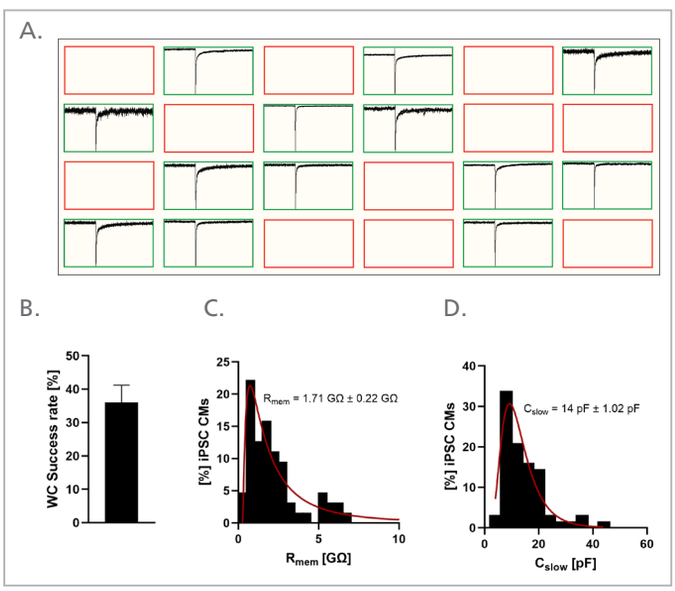


**Fig. 1: axoCells™ hiPSC-CMs perform well in physiological solutions on QPatch.** **A)** Plate view displaying $Na_V$ currents in half a measurement plate (24 experiment sites). Green and red are the experiments that pass and fail the quality filter, respectively. **B)** Experiment whole-cell (WC) success rate in percentage of 48 experiment sites ($R_{mem}$ > 200 MΩ and $C_{slow}$ > 4 pF). Data is mean ± SEM of three measurement plates. **C)** and **D)** Histograms of $R_{mem}$ and $C_{slow}$ values of the measured cell population ($N_{cells}$ = 65) together with the mean ± SEM values and best fit of the distribution (red line).

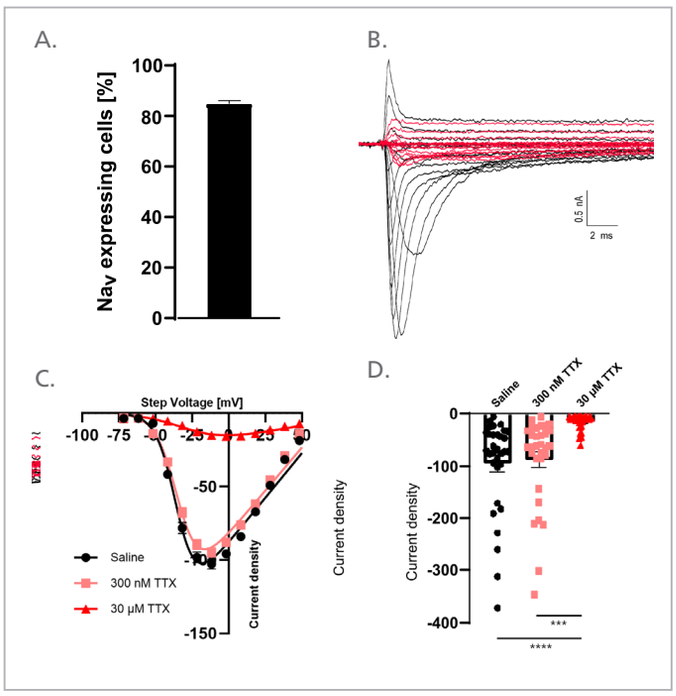


**Fig. 2: Recordings of $Na_V$ current in axoCells™ hiPSC-CMs in physiological solutions. A)** Percentage of successful experiments with $Na_V$ current. Data is mean ± SEM of three experiment plates. **B)** Representative $Na_V$ current trace in response to a voltage-step protocol before (black) and after (red) addition of 30 µM tetrodotoxin TTX. **C)** $Na_V$ current density vs. step voltage plot recorded in saline (black), 300 nM TTX (pink) and 30 µM TTX (red). Data points are mean ± SEM of 32 cells. **D)** Bar diagram of maximum $Na_V$ current density recorded in saline (black), 300 nM TTX (pink) and 30 µM TTX (red) showing significant block by 30 µM, but not 300 nM TTX. Statistical comparison was performed with a one-way ANOVA with Tukey's multiple comparison test, $p < 0.001$ (***) and $p < 0.0001$ (****).

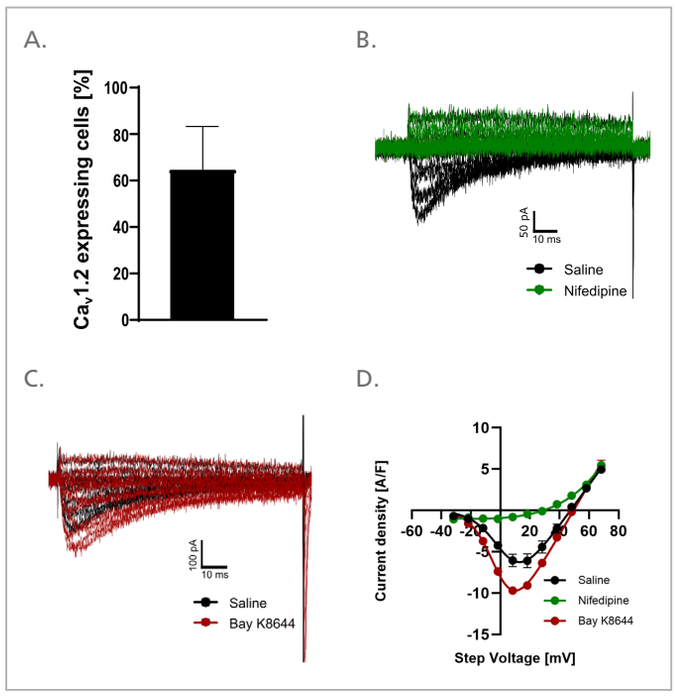


**Fig. 3: Recordings of $Ca_V$ current in axoCells™ hiPSC-CMs in physiological solutions. A)** Percentage of successful experiments with $Ca_V$ current. Data is mean ± SEM of three experiment plates. **B)** Representative $Ca_V$ current trace in response to a voltage step protocol before (black) and after (green) addition of a L-type $Ca_V$ channel blocker (10 µM nifedipine). **C)** Representative $Ca_V$ current trace in response to a voltage-step protocol before (black) and after (red) addition of an L-type $Ca_V$ channel agonist (0.3 µM bay K8644). **D)** $Ca_V$ current density vs. step voltage plot recorded in saline (black), 10 µM nifedipine (green) and 0.3 µM bay K8644 (red). Data points are mean ± SEM of 7 – 10 cells.

## Pharmacological and biophysical identification of the cardiac L-type $Ca_V1.2$ channel

In about 60 % of the hiPSC-CMs we recorded $Ca_V$ currents (Fig. 3A). The current was blocked by nifedipine and potentiated by bay K8644 (Fig. 3B and C), suggesting that the CMs contain the cardiac $Ca_V$ channel, namely the L-type $Ca_V1.2$ channel.

## Recordings of action potentials and modulation by $Ca_V1.2$ channel modulators

In about 60% of the hiPSC-CMs, we recorded paced action potentials, using the adaptive current clamp feature (Fig. 4A). This feature allows the user to adaptively inject current corresponding to a user-defined membrane potential before pacing. The threshold potential ($V_t$), peak potential ($V_p$), hyperpolarization potential ($V_h$) and action potential duration at 90% (APD90) were quantified (Fig. 4B). The potentials were relatively reproducible between the cells, with the APD90 being more variable, likely reflecting variation in ion channel expression

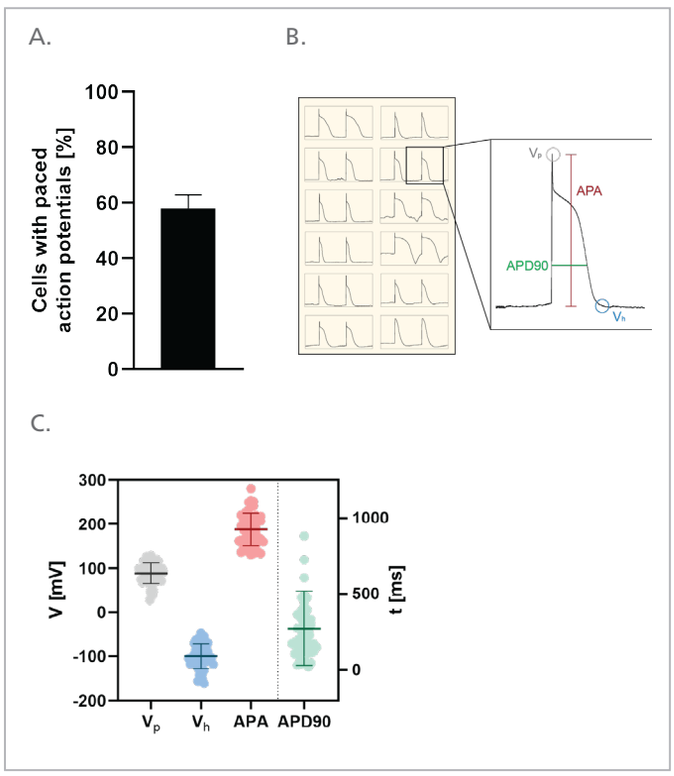


**Fig. 4**: **Recordings of paced action potentials in axoCells™ hiPSC-CMs in physiological solutions. A)** The percentage of successful experiments displaying paced action potentials. Data is mean ± SEM of 3 measurement plates. **B)** Paced action potentials from 12 individual hiPSC-CMs within a measurement plate (left) and an expanded action potential, exemplifying the extracted parameters: peak potential ($V_p$), hyperpolarization potential ($V_h$), action potential amplitude (APA) and action potential duration at 90% (APD90). **C)** Plot of extracted parameters, $V_p$ (grey), $V_h$ (blue), APA (red) and APD90 (green) for 52 individual iPSC CMs with the mean ± SD (solid lines).

The APD90 was quantified before and after addition of the $Ca_V1.2$ modulators, 0.3 µM bay K8644 (Fig. 5A) or 10 µM nifedipine (Fig. 5B). As expected, the APs were significantly prolonged when the $Ca_V1.2$ current was potentiated and shortened when the $Ca_V1.2$ current was blocked.

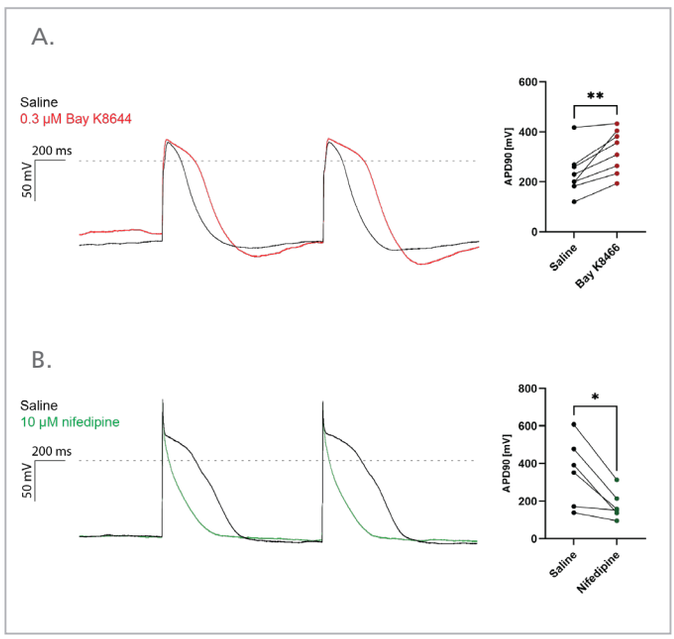


**Fig. 5**: **Recordings of APD90 single-concentration compound effects.** Paced action potentials and plot of APD90 values before (control, black traces and data points) and after addition of: **A)** 0.3 µM Bay K8644 (red trace and data) and **B)** 10 µM nifedipine (green trace and data). The statistical comparison was performed with a paired t-test, $p < 0.05$ (*) and $p < 0.01$ (**).

## Conclusion

In summary, it is possible to perform QPatch recordings of axoCells™ hiPSC-CMs in physiological ringers, yielding up to 40% whole-cell success rate, allowing the biophysical and pharmacological evaluation of cardiac voltage-gated ion channels ($Na_V1.5$ and $Ca_V1.2$) as well as paced action potentials.

The results exemplify APC as a robust and reliable technology for assessing the electrophysiological properties of hiPSC-CMs. We envision that the APC technology in combination with hiPSC-CMs can be used for disease modelling, cardiotoxicity and drug testing with the potential to improve the prediction of cardiac safety liabilities, reduce animal experimentation, and accelerate the translation of promising therapeutics to the clinic.

## Methods

**Cells and Culture:** Recordings were performed using axoCells™ human iPSC-derived ventricular cardiomyocytes (ax2508), kindly provided by AXOL. Cell culture was performed using Axol Bioscience reagents and protocols.

**Cell Suspension:** Cell suspension preparation was performed according to internal Sophion methods.

**Electrophysiology Solutions:** Contact Sophion for information regarding solutions.

**Electrophysiological Analysis:** All analysis was performed with Sophion Analyzer.

For information on dissociation procedures and solutions contact us at info@sophion.com.

Human iPSC-derived Ventricular Cardiomyocytes (CM) were kindly provided by

AXOL

Contact: operations@axolbio.com

## References


1. Takahashi, K. et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. **Cell** 131, 861–872 (2007).
2. Yu, J. *et al.* Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. **Science** (1979) 318, 1917–1920 (2007).



**Author:**

Kadla R. Rosholm, Sr. Research Scientist
Atanaska Velichkova, Research scientist



Sophion Bioscience A/S
info@sophion.com
sophion.com