# Application Note

Channel: $I_{Na}$, $I_{Ca}$, $I_K$
Cells: ES cell-derived cardiomyocytes
Tools: Patchliner®

# Recordings of Action Potentials in Mouse ES Cell-Derived Cor.At® Cardiomyocytes on Nanion's Patchliner®

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## Introduction

Axiogenesis is a provider of pure mouse embryonic stem cell-derived cardiomyocytes (Cor.At® (1)). These cardiomyocytes have been evaluated with Nanion's automated patch clamp platforms the Port-a-Patch® (2) and the Patchliner®. The aim of this study was to show that the Patchliner®, Nanion´s planar patch clamp device for increased throughput, can be used for studies investigating compounds which exhibit chronotropic or arrhythmic effects. In the voltage clamp mode voltage-dependent $Na^+$-, $Ca^{2+}$- and $K^+$-channel currents could be recorded (Fig. 1). As expected, action potentials could be elicited in the current clamp mode (Fig. 2). Effects of compounds on action potentials have been successfully demonstrated.

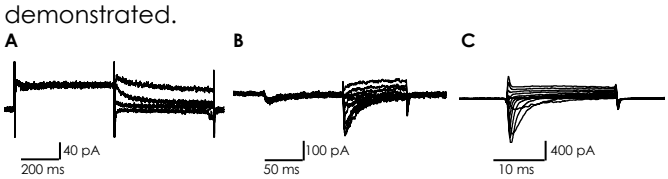


**Figure 1:**
Ion channel recordings in Cor.At® cardiomyocytes. **A** mERG currents showed approximately 50 pA outward current. **B** After inactivating T-type calcium currents with a pre-pulse, L-Type calcium current were elicited by voltage steps from -40 mV to +60 mV. **C** $Na^+$-channel mediated currents.

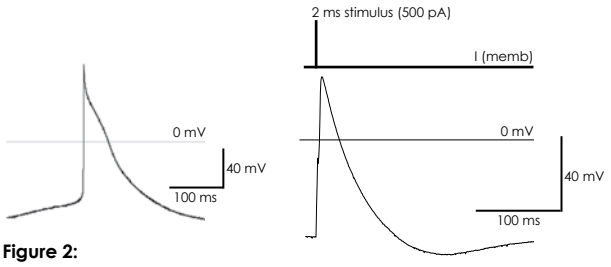


**Figure 2:**
Action potentials in Cor.At® cells, **A**, recorded with a conventional patch clamp rig (Dr. Davide Pau, Scottish Biomedical Ltd., Glasgow, UK), **B**, recorded with a Patchliner®. The action potential was elicited by a 2 ms pulse to 500 pA. Before and after the stimulation, the cell was kept at the holding current which corresponded to -80 mV.

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## Results

Action potentials recorded in the current clamp mode of Cor.At® cardiomyocytes were sensitive to the ion channel modulators Quinidine and Lidocaine (Fig. 3).

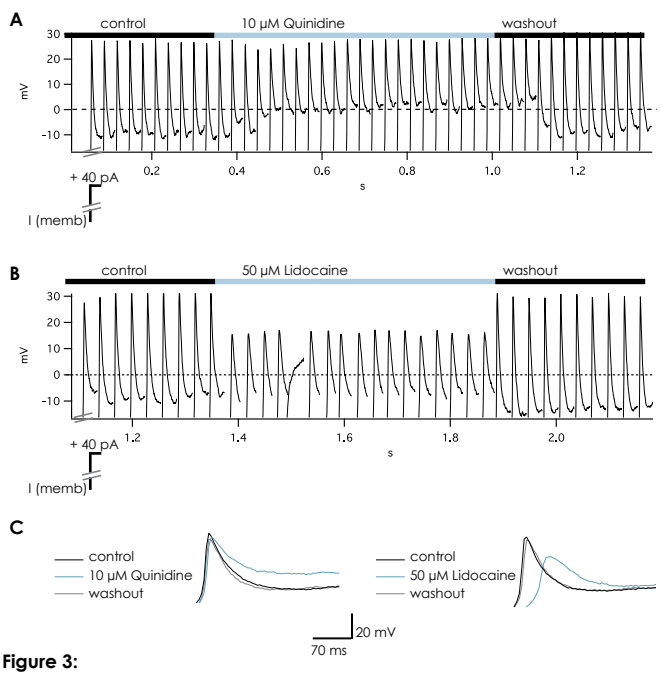


**Figure 3:**
Pharmacological experiments. **A** Action potentials in control conditions, in the presence of 10 µM Quinidine and after washout. **B** Action potentials in control conditions, in the presence of the $Na^+$-channel blocker Lidocaine (50 µM) and after washout. Same cell as in A. **C** Overlay of three traces from the recordings shown in A and B. The action potentials were elicited by depolarisation from a holding current $I_{(memb)}$ to +40 pA for 150 ms with a sweep interval of 10 s.

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# Application Note

The human Ether-a-go-go Related Gene (hERG) encodes a $K^+$-channel, which is responsible for the repolarising $I_{Kr}$ current in the human cardiac action potential. The hERG blocker Cisapride induced a change of the repolarisation (Fig. 4), which indicates functionality of the mouse analogue of hERG-channels in Cor.At® cardiomyocytes. The effect was non-reversible upon washout.

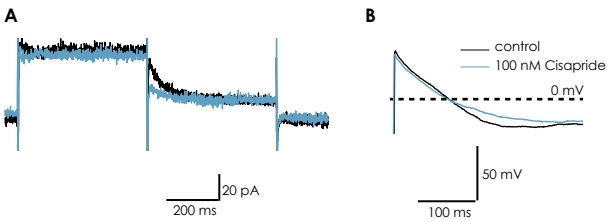


**Figure 4:**
Cisapride block of mERG. **A** mERG current in the voltage clamp mode, before and after the application of 100 nM Cisapride. **B** Action potential before and after the application of the hERG specific blocker. After application of 100 nM Cisapride, the repolarisation of the action potential was slower. This hints to a mERG channel block, since the hERG channel analog is, like in human action potentials, responsible for the repolarisation of action potentials in mouse fetal cardiomyocyte-like Cor.At® cells. The action potential was elicited by a 1 ms pulse to 200 pA. After the stimulation, the cell was kept at the basal level with no current injection (0 pA) for 500 ms. The holding current corresponded to -80 mV.

## Summary


Cor.At® cardiomyocytes display typical cardiac ion channel activity and action potentials. Both the hERG blockers Quinidine and Cisapride, and the $Na^+$-channel blocker Lidocaine, modulated the action potentials. The Quinidine and Lidocaine effects were reversible, the Cisapride effect was non-reversible.

The results demonstrate the presence of an array of ion channels in Cor.At® cardiomyocytes which in conjunction are capable of generating action potentials. Hence these stem cell-derived cardiomyocytes are a suitable alternative to primary cardiomyocytes in drug screening and safety testing.

In addition, these experiments demonstrate for the first time the suitability of a higher throughput planar patch clamp system, i.e. Nanion´s Patchliner®, for recording action potentials. This is possible because of the flexibility of Nanion's patch clamp systems allowing for a multitude of different experiments to be performed in both voltage and current clamp modes.

Cells expressing multiple ion channels and therefore able to elicit action potentials, as opposed to cell lines over-expressing a single ion channel subtype, better represent the physiological system. Thus, the Patchliner® in combination with Cor.At® cardiomyocytes form a powerful tool for ion channel research, drug screening and safety testing.


## Methods

Cor.At®

### Cells

Mouse embryonic stem cell-derived cardiomyocytes, ready to use and 99.9 % pure without contamination by other cell types (1).

### Patch Clamp Solutions

External solution for $Ca^{2+}$-channel recordings: 80 mM NaCl, 3 mM KCl, 10 mM $MgCl_2$, 35 mM $CaCl_2$, 10 mM HEPES ($Na^+$-salt)/HCl, pH 7.4. External solution in voltage clamp and current clamp recordings: 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM D-Glucose monohydrate, 10 mM HEPES /NaOH pH 7.4. Internal solution: 50 mM KCl, 10 mM NaCl, 60 mM KF, 20 mM EGTA, 10 mM HEPES /KOH, pH 7.2.

### Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. Protocol for ion channel recordings see (2).

### References


**(1)** Cor.At® cardiomyocytes from Axiogenesis AG, Cologne, Germany; www.axiogenesis.com, email: info@axiogenesis.com.

**(2)** Recordings of Action Potentials in Cor.At cells on Nanion´s Port-a-Patch®. www.nanion.de



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