cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

hERG Safety

Background Information

'The impressive list of drugs, already on the market or still under development that have been reported to adversely prolong repolarisation, makes it imperative to investigate any new chemical entity for this potential side effect before its first use in man.'

[1] Haverkamp W, Breithardt G, Camm AJ, Janse MJ, Rosen MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A and Shah R. (2000) *Eur Heart J* **21(15)**; 1216-31.

- The human ether-a-go-go related gene (hERG) encodes the inward rectifying voltage gated potassium channel in the heart (I_{Kr}) which is involved in cardiac repolarisation.
- Inhibition of the hERG current causes QT interval prolongation resulting in potentially fatal ventricular tachyarrhythmia called *Torsade de Pointes*.
- A number of drugs have been withdrawn from late stage clinical trials due to these cardiotoxic effects, therefore it is important to identify inhibitors early in drug discovery.
- The hERG Safety service is performed by our parent company, Evotec, and is a cell-based assay which employs the QPatch HTX System (Sophion Bioscience A/S) or the SyncroPatch 384PE (Nanion Technologies) as automated patch clamp electrophysiology measurements.
- The QPatch HTX and the SyncroPatch 384PE systems deliver high quality, accurate and sensitive data which are comparable with the traditional single cell patch clamp method.

Protocol

Instrument
QPatch HTX (Sophion Bioscience A/S) or SyncroPatch 384PE (Nanion Technologies)

Analysis Method
Electrophysiology

Cell Line
hERG stably transfected HEK293 cells

Patch Clamp Technique
Whole cell patch clamp (QPatch)
Perforated patch clamp (SyncroPatch)

Test Article Concentration
Typically, 0.1, 1, 10 µM, applied sequentially to the same cell (different range and number of concentrations available)

Number of Replicates
Each test article is usually tested in at least 2 cells

Quality Controls (QPatch)
0.1-0.5% DMSO (negative control)
E-4031 (positive control)
Rm > 100 MOhms
Pre-compound tail current > 0.2 nA

Quality Controls (SyncroPatch)
0.25-0.3% DMSO (negative control)
E-4031 (positive control)
Rseal ≥ 50 MOhms before compound addition (4-hole chips)
Peak current ≥ 0.2 nA before compound addition (4-hole chips)

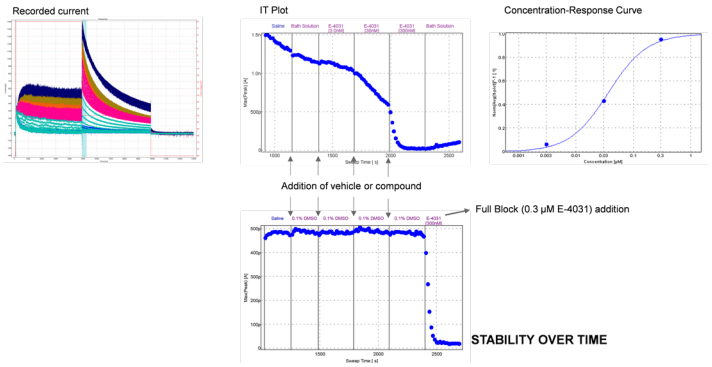
Test Article Requirements
100 µL of 10 mM solution

Data Delivery
Mean % inhibition and IC_{50} determination (if appropriate)

To date, electrophysiology remains the 'gold standard' method with which to characterise ion channel properties, as binding, flux and fluorescence assays only indirectly measure ion channel properties[1].

Figure 1

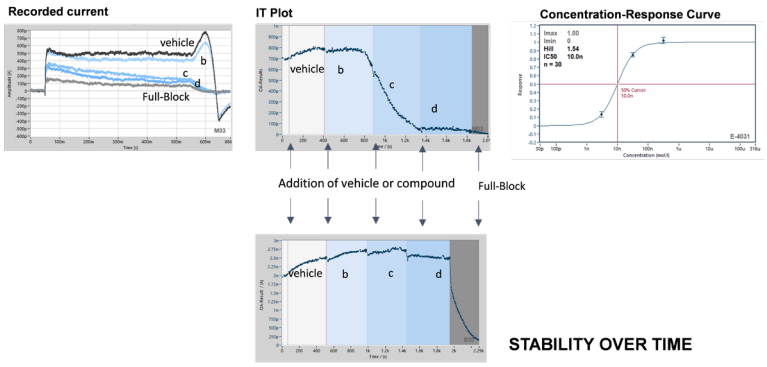
Example of current recording on the QPatch HTX.



The QPatch HTX is a high-throughput and automated electrophysiology platform. The QPatch performs up to 48 independent patch clamp experiments in parallel in a 48-channel chip array with microfluidic chambers (QPlate). QPatch HTX system uses the general principle of planar patch clamp: each chamber of the QPlate consists of two compartments containing extra- and intra-solutions, separated by a silicon-based glass planar substrate that has a micron-sized aperture. Cells in suspension are placed in the wells and interact with the glass to form stable giga-seal at the aperture. After giga-seal formation, whole-cell configuration is achieved by physical rupture of the cell-attached patch, then pre-programmed voltage-clamp steps are applied and recording begins.

Figure 2

Example of current recording on the SyncroPatch 384PE.



The SyncroPatch 384 Patch Engine (PE) automated patch clamp instrument simultaneously performs electrophysiology measurements for multiple single cells in specialised 384 well plates. After initiating the experiment, cell catching, sealing, perforated-cell formation, liquid application, recording, and data acquisition are performed sequentially. Initially, the chip is primed with appropriate extracellular and intracellular solutions. The suspended single cells, stored in a cell hotel reservoir with orbital shaking speed at 200 rpm, are aspirated from the reservoir, pipetted into the planar 384-well patch-clamp chip, and entrapped in the holes of the wells by an automatically applied vacuum. Seal generation, the establishment of the perforated cell mode and also the electrophysiological recordings are controlled by PatchControl 384. Once a stable patch has been achieved, recording commences in voltage-clamp mode.

Table 1

Comparison of Evotec hERG safety assays with literature values for known hERG inhibitors that cover a broad range of potencies.

	QPatch IC_{50}	SyncroPatch IC_{50}	Literature
E-4031	16-50 nM	10-30 nM	26.3 nM (QPatch)[2] 17 nM (SyncroPatch)[3]
Verapamil	0.28 µM	0.63 µM	0.2 µM (QPatch)[2] 0.59 µM (SyncroPatch)[3]
Quinidine	1.3-1.7 µM	0.73 µM	0.64 µM (QPatch)[2] 0.5 µM (SynchroPatch)[3]
Astemizole	5-14 nM	41 nM	28.1 nM (QPatch)[2] 19.8 nM (SyncroPatch)[4]
Bepridil	0.07-0.36 µM	0.16 µM	0.13 µM (QPatch)[2] 0.2 µM (Manual Patch Clamp)[5]

Data from the Evotec QPatch and SyncroPatch hERG inhibition assay are consistent with literature data as shown in Table 1. This includes the positive control compound E-4031, a known hERG inhibitor.

The hERG inhibition assay forms part of the CiPA cardiac ion channel panel for determining cardiac safety. Further cardiac ion channel assays are available as a non-GLP, CiPA-compliant panel from our parent company, Evotec.

References

[1] Haverkamp W *et al.* (2000) *Eur Heart J* **21(15)**; 1216-31
[2] Okada J *et al.* (2015) *Sci Adv* **1(4)**; e1400142
[3] Polonchuk L (2014) *Methods in Molec Biol* **1183**; 81-92
[4] Obergrussberger A *et al* (2016) *J Lab Autom* **21(6)**; 779-793
[4] Crumb WJ *et al* (2016) *J Pharmacol Toxicol Methods* **81**; 251-262