MANUAL PATCH CLAMP QUALITY + AUTOMATED PATCH CLAMP = CYTOPATCH™ INSTRUMENT

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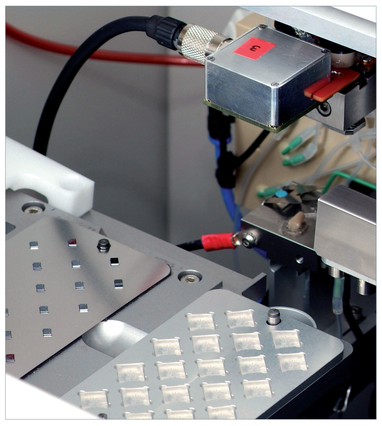
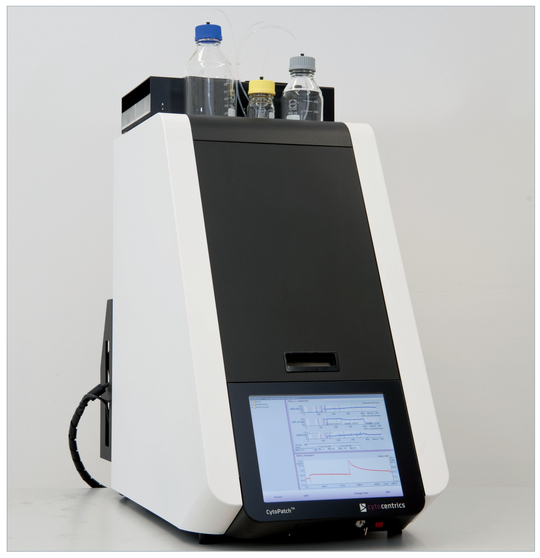
INTRODUCTION



The most important requirement in drug discovery is the reliability of data. In terms of ion channel screening, the manual patch clamp technique fulfils this in an excellent way. However, it has a very low throughput and high costs per data point. The automated patch clamp offers a much higher throughput and lower costs. But the data quality and the flexibility of the perfusion schemes of the manual patch clamp could not be completely reproduced. Cytocentrics addresses this demand with the CytoPatch™ Instrument. Its unique and patented Cytocentering Technology allows true gigaseal recordings without the need of seal driving chemicals. Two sophisticated liquid-handling systems enable a non-stop perfusion of the cell with either buffer or compound over several minutes. Furthermore, due to the flexible perfusion system, the fast application of agonists, as required for the investigation of fast desensitizing ligand ion channels, is possible with the CytoPatch™ Instrument. For example, the fast rise time (10 - 90 %) for the nicotinic acetylcholine receptor Alpha 7, of less than 5 ms, is shown. In summary, the CytoPatch™ Instrument offers the same flexibility and data quality known from the manual patch clamp. Simultaneously, it offers a higher throughput, lower costs and a higher standardisation of the measurement processes. Thus, it is an instrument that satisfies the contemporary needs and expectations on the patch clamp technique.



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BENEFIT OF THE CYTOPATCH™ TECHNOLOGY

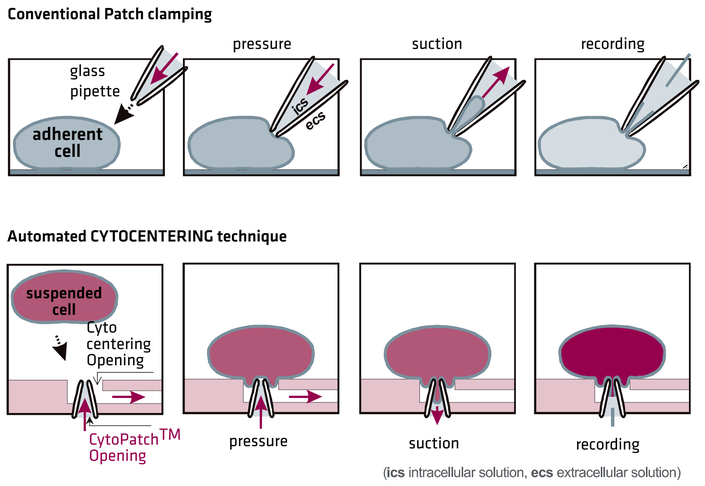
The CytoPatch™ Instrument has a unique technique which is well suited for the investigation of ligand gated as well as voltage gated ion channels.

- NOVEL CYTOCENTERING™ TECHNOLOGY ENABLES TRUE GIGASEALS
- AVOIDANCE OF SEAL-FORCING BUFFERS (NO FLUORIDE)
- PERMANENT BUFFER PERFUSION IN CONTROL PHASE
- PERMANENT COMPOUND PERFUSION
- NO CROSS-CONTAMINATION OF COMPOUND
- BEST RESULTS WITH „STICKY COMPOUNDS“

- SERIAL RESISTANCE: 10 MOHM
- SERIAL RESISTANCE COMPENSATION ☑
- LOW SYSTEM CAPACITY (< 10 PF)
- ULTRA-FAST APPLICATION FOR LGICS <10 MS
- GLP CONFORM (HLPC ANALYSIS ☑)
- FULLY AUTOMATED: SEVERAL HOURS WALK-AWAY TIME
- AUTOMATED DATA-EVALUATION

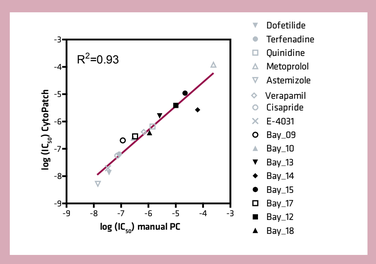
THE CYTOPATCH™ INSTRUMENT PROVIDES THE SAME HIGH DATA QUALITY AS THE MANUAL PATCH CLAMP TECHNOLOGY.

AUTOMATED PATCH CLAMP with real Giga Ohm seals



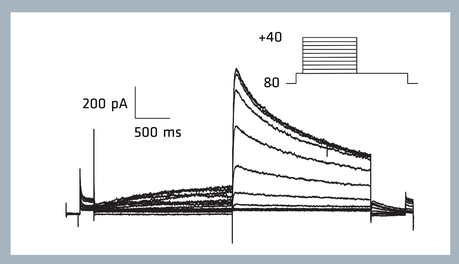
In contrast to other planar patch clamp platforms the unique CytoPatch™ Chip contains a real patch pipette that is surrounded by the Cytocentering opening. The latter is used to position the cell on the patch pipette. By application of precise pressure protocols through this patch pipette, gigaseals and the whole-cell break through are obtained. The sealed cell is then continuously perfused with extracellular buffer or test compound. This resembles the patch clamp process known from the manual patch clamp and results in the same high quality of recordings.

COMPARATIVE HERG STUDY

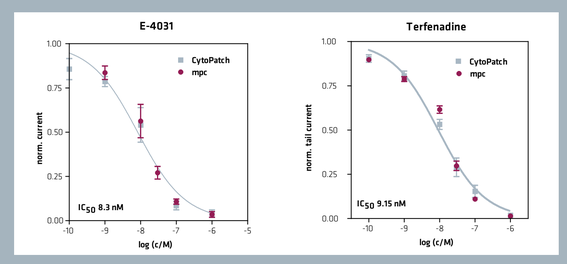


In a blinded study the effect of 18 compounds provided by Bayer Schering Pharma (Wuppertal) investigated with the CytoPatch™ Instrument was compared to manual patch clamp recordings performed at the Bayer site (Assay Drug Dev Technol. 2011 Jun 15. (Epub ahead of print)).

HERG SCREENING
in HEK293 Instant Cells

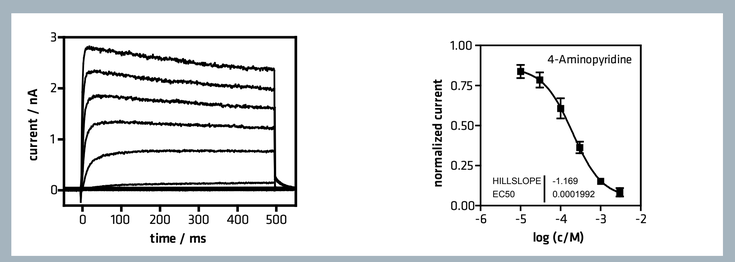


Representative hERG outward currents recorded from Instant HEK 293 cells stably expressing the hERG ion channel. Currents elicited by the voltage protocol shown top right.

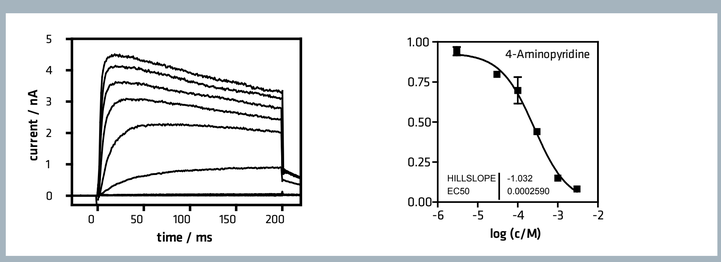


Concentration response relationship for the potent hERG inhibitors E-4031 (left) and terfenadine (right) determined with the CytoPatch™ Instrument (grey symbols and curves). In comparison data obtained with manual patch clamp recordings are depicted in red.

K_V1.5 AND K_V1.3 SCREENING WITH THE CYTOPATCH™ INSTRUMENT

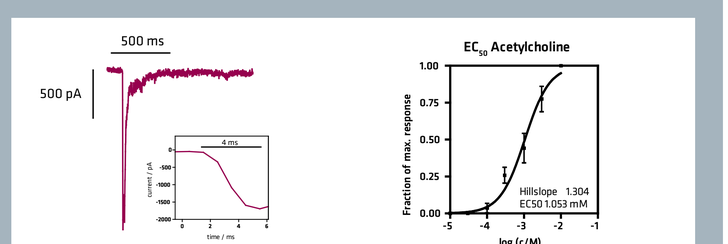


Whole-cell K_V1.5 currents stably expressed in CHO-K1 cells recorded with the CytoPatch™ Instrument (left). Concentration-response relationship for the inhibition of K_V1.5 currents by the K channel blocker 4-AP (right).



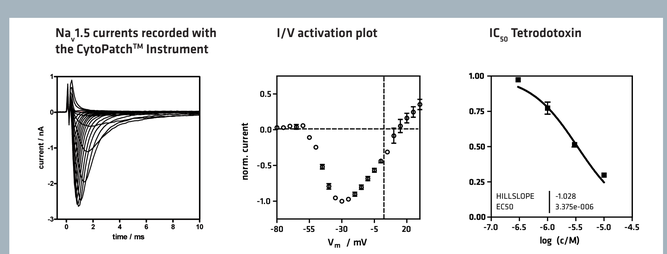
Whole-cell K_V1.3 currents stably expressed in CHO-K1 cells recorded with the CytoPatch™ Instrument (left). Concentration-response relationship for the inhibition of K_V1.3 currents by the K channel blocker 4-AP (right).

FAST LIGAND GATED ION CHANNELS



Single current trace of human nicotinic acetylcholine receptor alpha 7, stably expressed in CHO-K1 cells, measured with the CytoPatch™ Instrument (left) and concentration response curve for the agonist acetylcholine measured with the CytoPatch™ Instrument (right).

CARDIAC SODIUM CHANNEL Na_V1.5



Current voltage relationship for human Na_V1.5 stably expressed in Hek 293 cells, measured with the CytoPatch™ Instrument. Cells were clamped from a holding potential of –100 mV in 10 ms pulses to test voltages from –70 to +35 mV in 5 mV increments. Concentration-response relationship for the inhibition of Na_V1.5 by Tetrodotoxin (right)