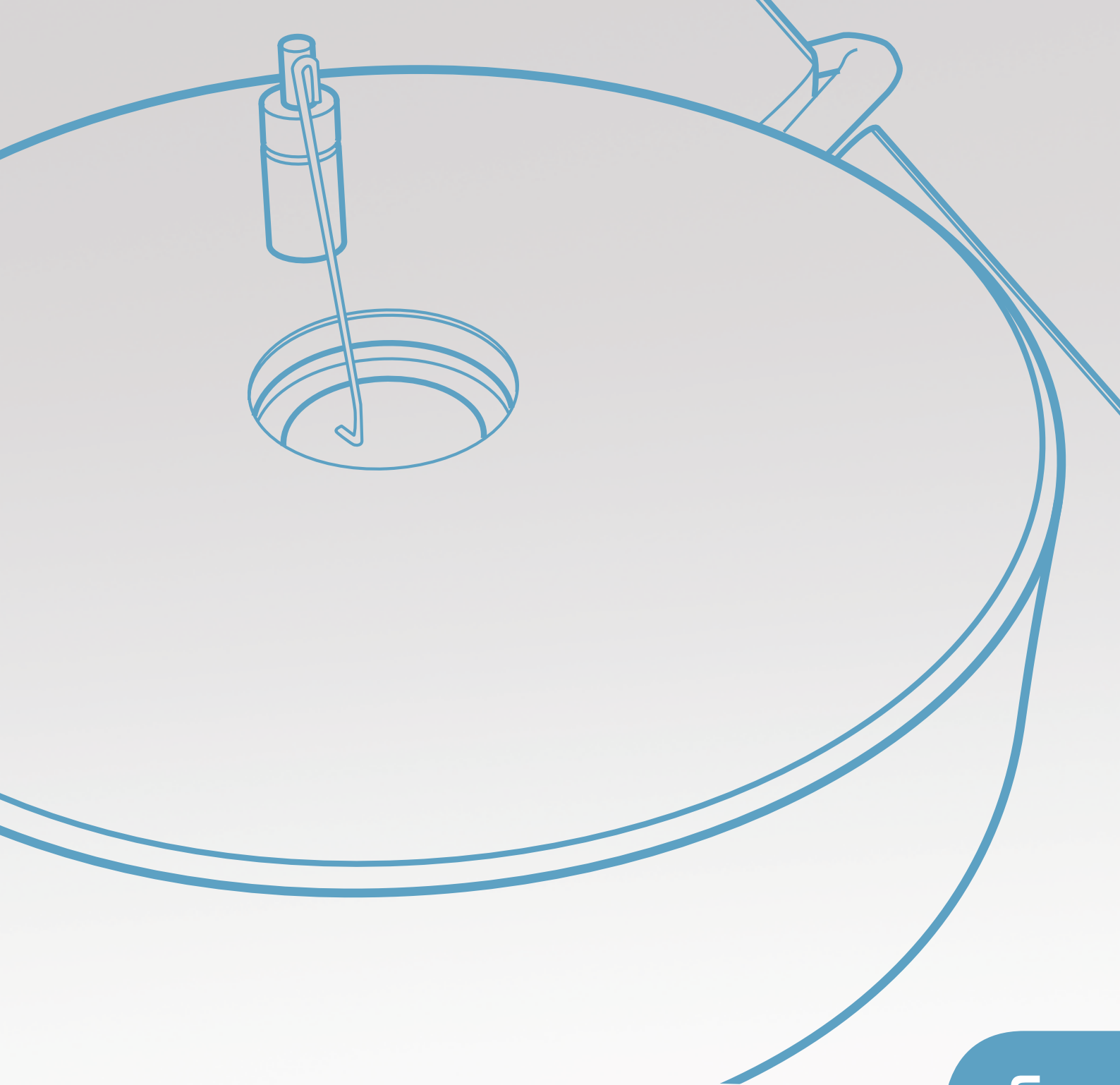
Internal Perfusion

Port-a-Patch® – Reaching inside the cell.

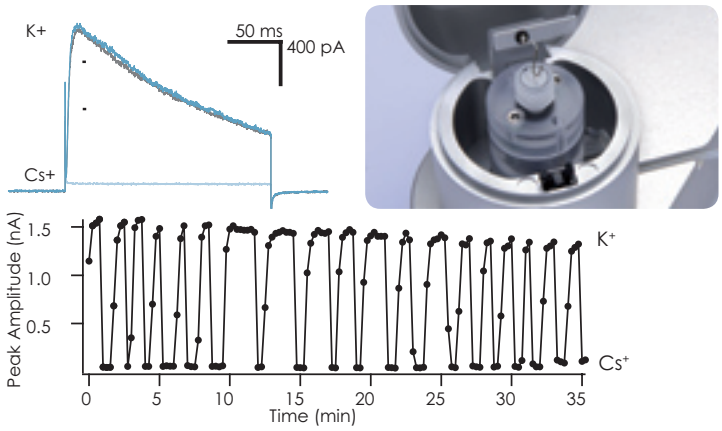
- **Robust and reliable internal solution exchange**
- **Complete exchange of solutions in seconds**
- **Continuous internal perfusion possible**
- **Data recordings possible during solution exchange**



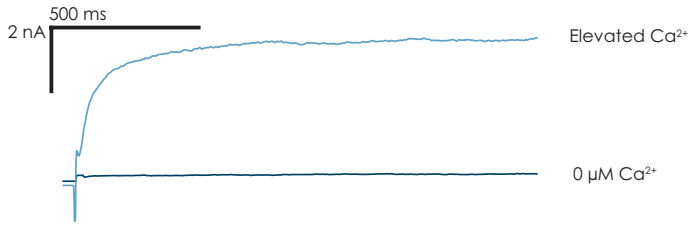
Internal Perfusion

Nanion's planar patch clamp chips offer the unique possibility to perfuse the intracellular side of the membrane. This aspect is exploited with the Internal Perfusion System for the Port-a-Patch®. Up to eight different solutions can be perfused inside the cell, allowing dose response analysis of compounds acting on the cytosolic portion of the ion channel. Second messengers and metabolites can also be added internally to investigate how ion channel function is affected. Solutions are exchanged within seconds, and the ion channel modulation can be continuously monitored during the solution exchange.

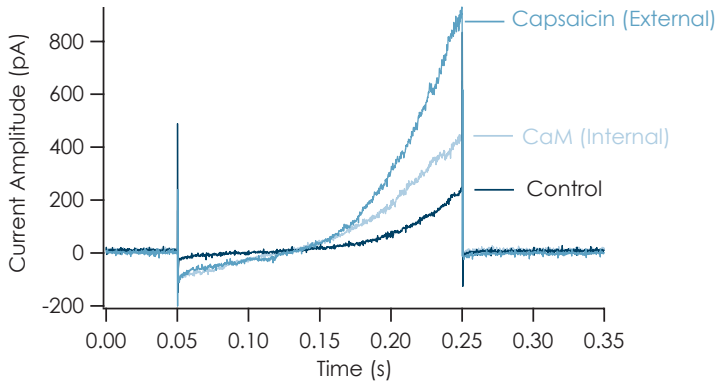
The Internal Perfusion System is an add-on for the Port-a-Patch®. Combined with Nanion's Perfusion System it can be operated either manually, or fully computer controlled. In this way, data acquisition can be synchronized with solution exchange, and traces are automatically tagged with the compound information.



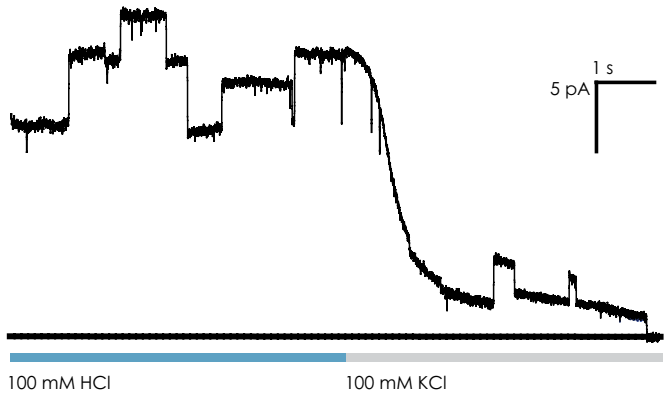
Stable recordings during internal perfusion. $K_V1.3$ currents (blue), endogenously expressed in Jurkat cells, were rapidly blocked by internal perfusion of Cs^+ (light blue), and fully recovered after washout with K^+ (grey). Internal solution replacement was repeated 19 times and the recording was stable for over 35 minutes, as shown in the lower graph.



Internal application of second messengers. Currents mediated by BK channels expressed in CHO cells were studied using the Internal Perfusion System. Currents were elicited by a voltage step from -80 mV to +80 mV before and after adding an internal solution containing a higher concentration of free Ca^{2+}.



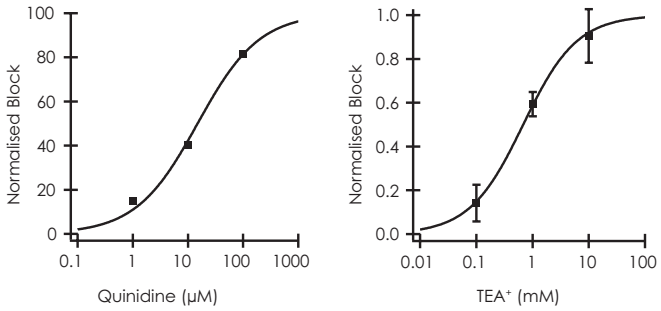
Internal application of Ca^{2+}-calmodulin to TRPV1.
TRPV1 currents were elicited by the external application of 20 µM capsaicin. After capsaicin activation, the currents were partially blocked by the internal application of Ca^{2+}-calmodulin (50 µM Ca^{2+}/500 nM CaM). TRPV1 channels were expressed in CHO cells.



Rapid internal exchange.
Switching of internal solutions during gramicidin recordings from a lipid bilayer was obtained within seconds. A lipid bilayer was formed using giant unilamellar vesicles. Gramicidin was incorporated into the lipid bilayer. Currents were recorded at a holding potential of +150 mV. The internal solution was switched from HCl to KCl, resulting in lower channel conductance.

Pharmacological experiments.

$K_V1.3$ current was blocked by the internal application of increasing concentrations of quinidine (left) or TEA^+ (right). The graphs show the Hill plots for IC_{50} determination. For quinidine the IC_{50} was determined as 15 µM (literature value external application 14 µM) and for TEA^+ the IC_{50} was determined as 0.9 ± 0.3 mM (n = 3) (literature value internal application 0.6 mM).



Product Number	Specification	Size & Weight
02 2001	**Internal Perfusion with Perfusion System** *(02 1002)* • Valve Control Panel (analog, digital and USB-ports for computer control) • Internal Perfusion Chamber with housing • Internal Perfusion starter kit: electrodes, solutions, tubings and other accessories • Perfusion System starter kit with 50 ml syringes, Reagent Kit, tubing, connectors, waste bottle *etc.* • Stand with syringe holder	Valve Control Panel Size (l x w x h): 40 x 5.8 x 13 cm Weight: 2.45 kg Internal perfusion Size (l x w x h): 7.0 x 7.0 x 10 cm Weight: 460 g
02 2002	**Internal Perfusion without Perfusion System*** • Internal Perfusion Chamber with housing • Internal Perfusion starter kit: electrodes, Reagent Kit, tubings and other accessories * Only compatible with Nanion's Perfusion System	Internal perfusion Size (l x w x h): 7.0 x 7.0 x 10 cm Weight: 460 g