Application Report:

# HEK293-$K_{Ca}$

## Calcium-activated potassium channels


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Sophion Bioscience A/S, Baltorpvej 154, DK-2750 Ballerup, Danmark
Phone: + 45 44 60 88 00 Fax: +45 44 60 88 99 E-mail: info@sophion.dk www.sophion.com

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## Introduction

Performing automated patch clamp experiments with different intracellular solutions is somehow difficult. Using QPatch with its multi well reservoir is it possible to test eight different solutions on the same cell line.

In this study the multi well reservoir has been used for experiments on the voltage-dependent large-conductance $Ca^{2+}$-activated $K^+$ channels, often referred to as BK channels. A human embryonic kidney cell line (HEK293) expressing the BK channel was used.

BK channels are large conductance calcium and voltage gated potassium channels, which allow potassium flux when activated with intra cellular calcium and/or membrane potential (Strøbæk *et al*., 1996).

## Materials & Methods

| | |
|---|---|
| Phosphate buffer solution (PBS) | 14190 Invitrogen |
| Trypsin/EDTA | 15400 Invitrogen |
| Tryphan Blue | T8154 Sigma |
| **Serum-free Media** | |
| CHO-S-SFM II | 12052 Invitrogen |
| HEPES | 15630 Invitrogen |
| Soy bean trypsin inhibitor | T6522 Sigma |
| Penicillin/Streptomycin (P/S) | 15140 Invitrogen |
| **Culture Media** | |
| D-MEM media | 32430 Invitrogen |
| Foetal Bovine Serum (FBS) | F7524 Sigma |
| Penicillin/Streptomycin (P/S) | 15140 Invitrogen |
| **Ringer's solution** | |
| Intracellular solutions | In mM (all four solutions, pH=7.2, mOsm ≈ 295)<br>**0.00001**: 0.965 $CaCl_2$, 1.785 $MgCl_2$, 30/10 KOH/EGTA, 120 KCl, 10 M HEPES<br>**0.0003**: 5.17 $CaCl_2$, 1.42 $MgCl_2$, 30/10 KOH/EGTA, 110 KCl, 10 M HEPES<br>**0.003**: 1.085 $CaCl_2$, 5.54 $MgCl_2$, 3/1 KOH/EGTA, 27/9 KOH/NTA, 120 KCl, 1 M HEPES<br>**0.01**: 1.37 $CaCl_2$, 5.41 $MgCl_2$, 3/1 KOH/EGTA, 27/9 KOH/NTA,120 KCl, 1 M HEPES |
| Extracellular solution | In mM: 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 145 NaCl, 4 KCl, 10 Glucose. pH 7.4; mOsm ≈ 310 |

Cells were cultured according to the Sophion standard HEK SOP. Passaged three times a week and harvested with trypsin and transferred to a serum-free media for experiments.

The voltage protocol used was an IV protocol (-120 to +120 mV), with a holding potential at -80 mV (Figure 1, left).

# Results

Four intra cellular Ringers with different concentrations of free calcium were tested on the HEK-BK cell line (10. 3. 0,3 and 0,01 µM free calcium). Seals were obtained in physiological Ringers solutions. The extra cellular solution was changed during experiments. NaCl → KCl → NaCl → TEACl → NaCl.

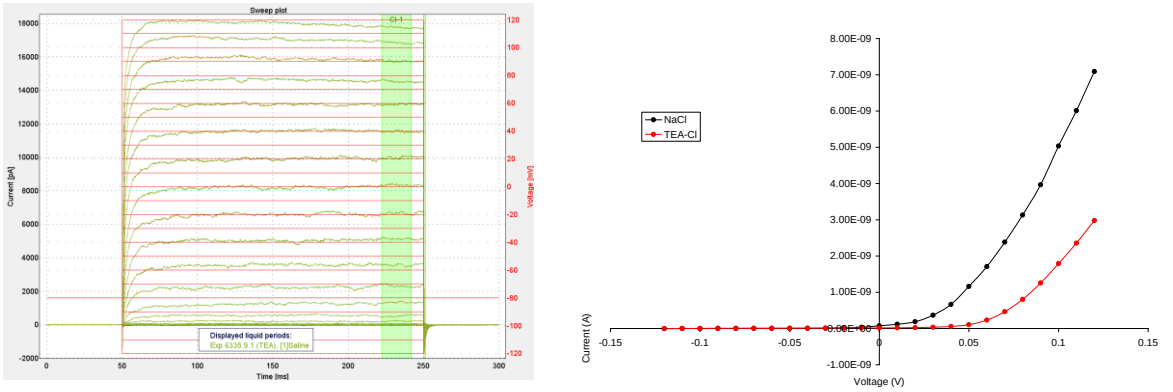


Figure 1. Left: Raw sweep in physiological Ringers solutions (asymmetrical), and Right: IV relation in control situation (black) and with 5 mM TEA-Cl (red)

The raw current traces from the BK channel are shown in Figure 1 (left). In Figure (right) it can be seen that the current can be partly blocked with 5 mM tetraethyl ammonium chloride (TEA-Cl) as expected.

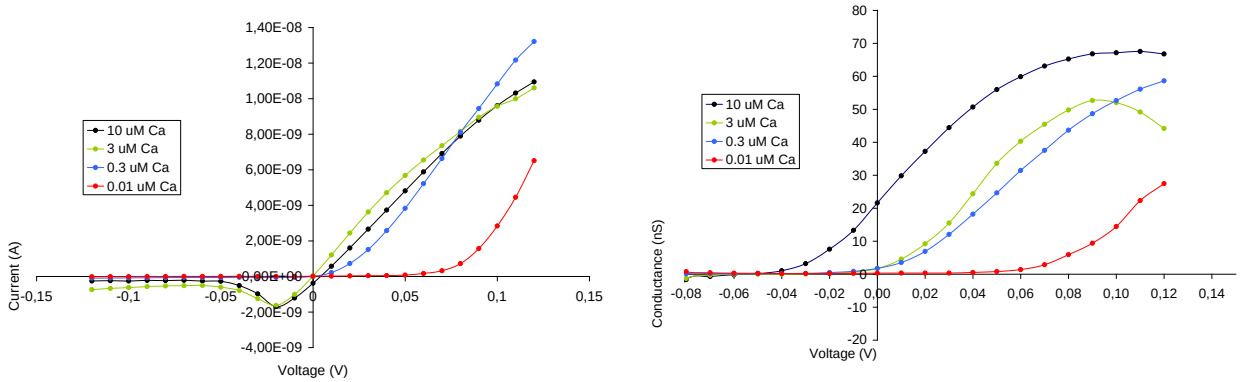


Figure 2. Left: IV relations with different concentrations of intra cellular calcium in symmetrical Ringers solutions. Right: GV relations with different concentrations of intra cellular calcium in physiological Ringers solutions (asymmetrical)

Figure 2 (left) shows the calcium, and voltage dependence of BK channel. With a high concentration of calcium, an inward current is obtained at negative potentials as expected. This is also what one would expected when the reversal potential ENernst(K) is at 0 mV. The conductance/potential plot (GV-plot) in Figure 2 (right) is

made in physiological Ringers solutions (asymmetrical) and clearly shows that conductance is highly calcium dependent.

## Conclusion

In these studies four different concentrations of free intracellular calcium was simultaneously tested on HEK293 cells expressing the BK channel using QPatch. The obtained data is in accordance with previously published data (Strøbæk *et al.*, 1996).

Controlling free calcium in electrophysiological experiments is a great task, since current change so rapid with changing calcium concentrations. Using different Ringers solutions in one job is unique for QPatch and shows the flexibility of QPatch. Using QPatch with different intracellular Ringers solutions it is possible to track the different Ringers solutions through the whole data analysis and QPatch thereby deliver a final report on which Ringers performed best. The feature is very useful for Ringers solutions optimization and as it was used in this study, for testing different compositions of intracellular Ringers solutions.