

B'SYS GmbH

# HEK Cav3.2 Cell Line

Specification Sheet

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## 1 BACKGROUND

### 1.1 B'SYS's HEK Cav3.2 Cells

B'SYS has designed a HEK Cav3.2 cell line. The human Cav3.2 cDNA was cloned and transfected into HEK cells and then the functional properties of the Cav3.2 channels validated by means of the patch-clamp technique. Results are outlined in section 3.

## 2 PRODUCT SHIPMENT

### 2.1 Product Format

HEK cells stably transfected with recombinant human Cav3.2 channel:

- 0.5 mL aliquots of frozen cells at approximately  $1.0 - 3.0 \times 10^6$  cells/mL
- Cells are frozen in complete medium with 10% DMSO

### 2.2 Mycoplasma Certificate

B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

## 3 VALIDATION OF HEK Cav3.2 CELLS

HEK Cav3.2 cells were tested by manual and automated patch-clamping (Q-Patch).

### 3.1 Electrophysiology

Cav3.2 currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) CsF 135, NaCl 10, HEPES 10, EGTA 5, pH (KOH) 7.30. After formation of a GΩ seal between the patch electrodes and individual Cav3.2 stably transfected HEK cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established inward calcium currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to +60 mV in 10 mV increments of 50 ms duration (Fig. 1). The voltage pulses were run at intervals of 10 s.

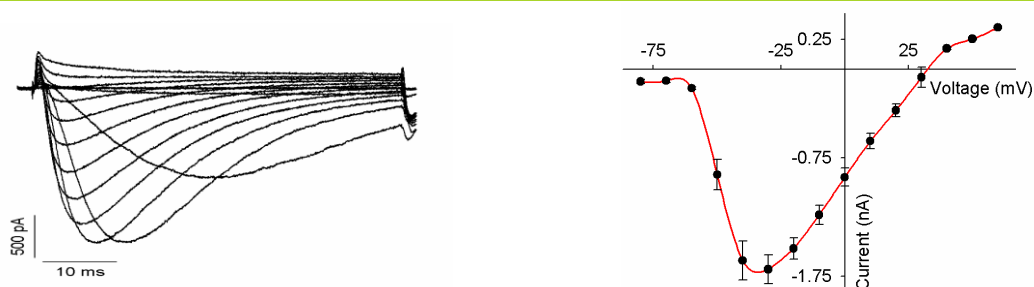
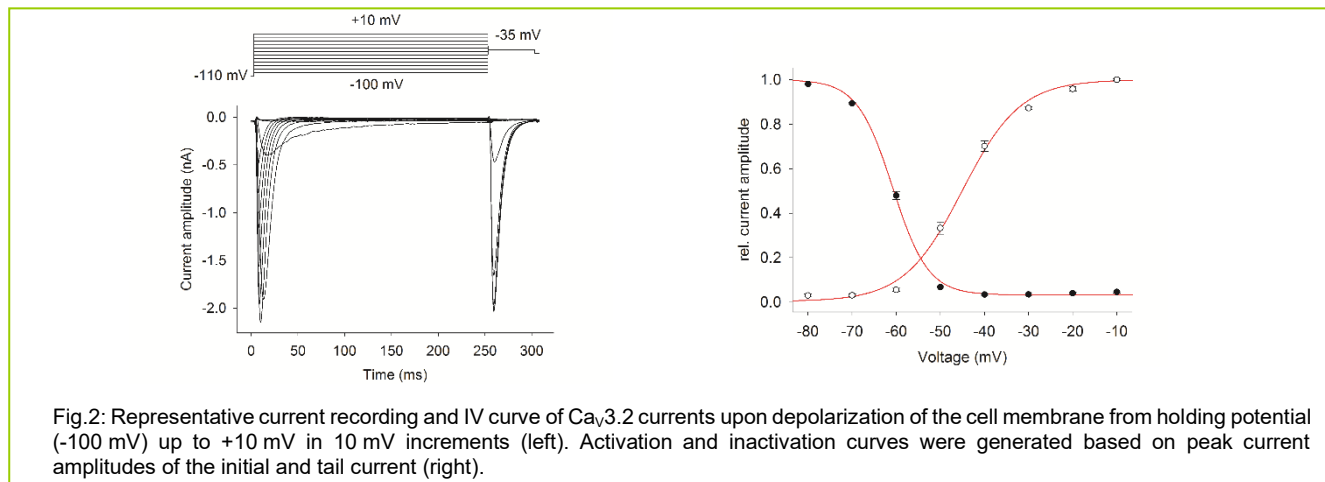


Fig. 1: Representative current recording and IV curve of Cav3.2 currents upon depolarization of the cell membrane from holding potential (-80 mV) up to +60 mV in 10 mV increments. No inward calcium currents were recorded in untransfected cells (data not shown).

Based on the IV curve the activation curve was generated. The  $V_{0.5}$  was determined to be  $-45.00 \pm 1.65$  mV.,  $k$ :  $6.45 \pm 0.30$  (n=4). For the inactivation curve a  $V_{0.5}$  of  $-60.78 \pm 0.28$  mV was found.



### 3.2 Mibefradil

Mibefradil, which is known as a T-Type  $Ca_v$  blocker, was used as positive control to validate the pharmacological profile of the HEK  $Ca_v3.2$  cells. The effect of Mibefradil was first measured at 10  $\mu$ M, which blocked  $Ca_v3.2$  currents almost completely ( $9.1 \pm 2.3$  % relative current amplitude, mean  $\pm$  SEM of 5 cells), holding potential -80 mV). When the cells were exposed to 0.01, 0.1, 1.0 and 10  $\mu$ M Mibefradil, the steady-state level relative current amplitudes revealed values of  $84.7 \pm 3.2\%$ ,  $57.3 \pm 4.0\%$ ,  $19.1 \pm 2.6$  and  $9.1 \pm 2.3\%$ , respectively (mean  $\pm$  SEM of 3 cells). The inhibition curve (Fig. 3) was best fitted with an  $IC_{50}$  value of 143.7 nM ( $V_H=-80$  mV). Using automated patch-clamping a  $IC_{50}$  of 1.55  $\mu$ M ( $V_H: -100$  mV)

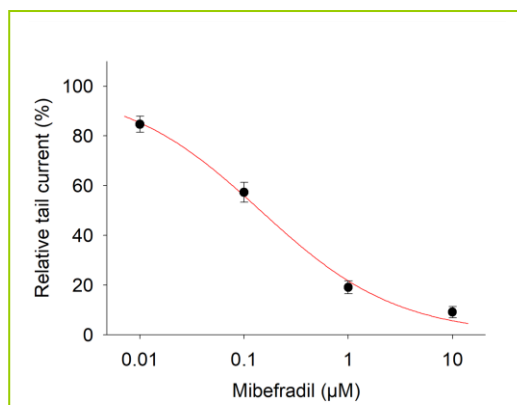


Fig. 3:  $Ca_v3.2$  current inhibition curve of Mibefradil.

## 4 Cav3.2 SEQUENCE

### 4.1 Cav3.2

Cloned cDNA sequence of Cav3.2 (CACNA1H) subunit was error-free and is encoding for NP\_066921:

```
MTEGARAADAEVRVPLGAPPPGPAALVGAESPESPGAPGREAEERGSELGVSPSESPAERGAELGADEEQRVYPYPAL
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LTVPQCPLKPGSPSATPAPGGGADDPV
```

## 5 CONTACT INFORMATION

### 5.1 Contact Address for Technical Support & Ordering Information

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