PRODUCT DATASHEET

PrecisION™ hKv3.1-CHO Recombinant Cell Line

Catalog Number: CYL3043

PRODUCT DESCRIPTION
Recombinant CHO-K1 cell line expressing the human voltage-gated potassium channel Kv3.1 (accession number NM_004976; Shaw-related subfamily, member1, KCN1).

ASSOCIATED PRODUCTS
The PrecisION™ hKv1.5-CHO Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:

- CYL3043SS PrecisION™ hKv3.1-CHO Single Site License
- CYL3043TS PrecisION™ hKv3.1-CHO Two Site License
- CYL3043MS PrecisION™ hKv3.1-CHO Multiple Site License

CONTENTS
2 x 1 mL aliquots containing 2.66 x 10⁶ cells/mL in 7.5% DMSO at passage 8.

STORAGE
Vials are to be stored in liquid N₂.

WARNINGS
For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneetisesti muutettuja organismieja.
Denna produkt innehåller genetiskt ändrade organismer.

MYCOPLASMA TESTING
The cell line has been screened using the ELISA based Mycoplasma Detection kit (Roche) and by a PCR VenorGem kit (Minerva Biolabs) to confirm the absence of Mycoplasma species.
FUNCTIONAL VALIDATION
CHO-K1 cells expressing hKv3.1 were characterized in terms of their pharmacological and biophysical properties using whole-cell patch clamp techniques and IonWorks™ HT. Using whole-cell patch clamp techniques the peak I/V relationship was linear and had a high threshold for current activation (-20 mV), typical of hKv3.1 currents. Riluzole and TEA have been shown to block hKv3.1 currents in the micromolar range. For example, application of TEA (200 µM) or riluzole (300 µM) blocked currents within a similar concentration range; 39.6 ± 3.1 % inhibition (peak current, n=5) and 39.9 ± 6.0 % inhibition (end current, n=5) respectively. Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 31 passages. The mean peak hKv3.1 outward current amplitude on stepping to +30 mV from a holding potential of -80 mV was consistently between 3–4 nA. Furthermore, of the cells that sealed, the percent of cells expressing hKv3.1 currents (i.e. currents >500 pA) was consistently between 98–100 %.

RECOMMENDED CULTURE CONDITIONS
Recommended culture conditions and standard operating procedure are provided with the product.

INTRODUCTION
Kv3.1 mRNA is abundantly expressed in auditory brain stem neurons that are able to spike at high frequencies. It has a high threshold of activation and rapid activation/deactivation kinetics (Wang et al., 1998). Evidence for functional Kv3.1 activity in such neurons can be derived from the rapidly activating and deactivating potassium currents seen here, that activate at a high-voltage threshold similar to that of heterologously expressed Kv3.1 (Gan and Kaczmareck, 1998). There are indications that the phosphorylation state in rat Kv3.1 may play a part in the ability of auditory neurons to adjust to the ambient acoustic environment (Song et al., 2005). It is thought that hKv3.1 subunits are likely to form homomultimers in lymphocytes and therefore could be a target for the development of novel immunosuppresants. (Grissmer et al., 1994).
FUNCTIONAL VALIDATION
Electrophysiological Properties of the hKv3.1 Current.

Conventional Whole-Cell Patch Clamp Electrophysiology.

CURRENT/VOLTAGE RELATIONSHIP:
Cells were voltage-clamped at a holding potential of –80 mV and depolarizing steps of 1 s applied in 10 mV increments to +30 mV. The currents elicited display features indicative of Kv3.1 (Figure 1) including large outward currents with a threshold of activation at around –20 mV and a linear I/V relationship (Figure 2). Slow inactivation at some potentials during the long depolarizing pulse was also evident (Grissmer et al., 1994, Kanemasa et al., 1995, Friederich et al., 2003).

Figure 1. I/V Relationship – Typical Current Trace.

Upper panel: Sample current traces from a cell held at –80 mV and with currents evoked by consecutive 1 s steps in 10 mV increments from –80 mV to +30 mV.

Lower panel: Voltage protocol.
Figure 2. Mean I/V Relationship.
Cells were held at −80 mV and currents evoked by 1 s steps in 10 mV increments from −80 mV to +30 mV (as example in Figure 1). Mean peak current amplitudes are plotted against voltage to outline the I/V characteristics (n=3).
Pharmacology – Riluzole:
The neuroprotective drug riluzole blocks hKv3.1 currents in the micromolar range. The drug appears to be blocking during the depolarizing pulse (Figure 3) and suggests open or activated block in agreement with published findings (Ahn et al., 2005). The mean percent inhibition of current after application of 300 µM was 39.9 ± 6.0% at the peak of the pulse and 82.6 ± 3.6% at the end of the depolarizing pulse (Figure 4).

Figure 3. Effect of riluzole on hKv3.1 currents.

Upper panel: Sample current trace for a cell held at –80 mV and current evoked by 1 s steps to –10 mV in control buffer conditions and in the presence of 300 µM Riluzole.

Lower panel: Voltage protocol.

Figure 4. Effect of riluzole on peak and end hKv3.1 currents.
The mean percent inhibition of current by 300 µM riluzole at the peak and at end of the depolarizing pulse (see Figure 3), n=5.
**Pharmacology – Tetraethylammonium chloride (TEA):**
A characteristic of Kv3.1 is its high sensitivity to TEA relative to other voltage gated potassium channels (Grissmer et al., 1994). TEA blocked the hKv3.1 channels in a dose dependent manner, with 2 µM inhibiting the peak current by 2.1 ± 1.2 %, 200 µM by 39.6 ± 3.1 % and 2 mM by 81.4 ± 2.0 % (Figures 5 and 6). This inhibition data compares well with published findings (Grissmer et al., 1994, Jarolimek et al., 1995, Friederich et al., 2003, Quinn and Begenisich, 2006.).

**Figure 5. Effect of TEA on hKv3.1 currents.**
**Upper panel:** Sample current traces for a cell held at –80 mV with currents evoked by 1 s steps to –10 mV in control buffer (black trace) or perfused with 20 µM (red), 200 µM (blue) and 2mM TEA (cyan). The red dotted line represents zero current level.
**Lower panel:** Voltage protocol.

**Figure 6. Effect of TEA on hKv3.1 currents.**
Mean percent inhibition of peak current by 20 µM, 200 µM and 2 mM TEA (n=5).
Stability of hKv3.1-CHO K1 Cell Line.

IonWorks™ HT Electrophysiology.

The hKv3.1-CHO K1 cell line has stable expression for >31 passages.

Functional channel expression, defined as cells expressing peak outward hKv3.1 current of ≥500 pA on stepping from a holding potential of -80 mV to +30 mV for 4 s, was monitored using IonWorks™ HT. This data and the mean current amplitudes are shown in Figure 7.

**Figure 7. Stability of expression over passage.**
The upper panel shows the percentage of cells expressing a mean peak current ≥500 pA (+30 mV) at cell passages 4, 11, 16, 21, 27 and 31. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles – out of 32 cells at passage 4 and out of 192 cells for all other passages).
Vector:

**Polylinker:** CMV-BamHI-NotI-**hKv3.1**-EcoRI-IRES-neo

**hKv3.1 Sequence:**
The sequence of the cDNA used to make this cell line contains one silent mutation with respect to the accession number (NM_004976) TGT-TGC (Cys) - at position 1258-1260.
REFERENCES


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