PRODUCT DATASHEET

PrecisIOn™ hKv2.1-CHO Recombinant Cell Line

Catalog Number: CYL3022

PRODUCT DESCRIPTION
Recombinant CHO-K1 cell line expressing the human voltage-gated potassium channel Kv2.1 (accession number NM_004975).

ASSOCIATED PRODUCTS
The PrecisIOn™ hKv2.1-CHO Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:
- CYL3022SS  PrecisIOn™ hKv2.1-CHO Single Site License
- CYL3022TS  PrecisIOn™ hKv2.1-CHO Two Site License
- CYL3022MS  PrecisIOn™ hKv2.1-CHO Multiple Site License

CONTENTS
2 x 1 mL aliquots containing 1.56 x 10^6 cells/mL in 7.5% DMSO at passage 12.

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 geneettisesti muutettuja organismeja.
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 hKv2.1 were characterized in terms of their biophysical and pharmacological properties using whole-cell patch clamp techniques. The mean current amplitude at +60 mV was 5.63 ± 0.86 nA (n=11). The threshold for current activation was between -20 and -30 mV. These properties are characteristic of hKv2.1 currents described in the literature. The cell-line is shown to be expressing hKv2.1 current since it is blocked by low mM concentrations of the potassium channel blockers 4-aminopyridine (10 mM; 73.9 ± 1.7 %, n=3) and tetraethylammonium (10 mM; 76.0 ± 0.1%, n=3-4). Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 26 passages: 76% of cells expressed outward current >500 pA at passage 26 (n=117). The mean current amplitude of these cells was 2420 ± 120 pA.

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

FUNCTIONAL VALIDATION
Electrophysiological Properties of the hKv2.1 current.

Conventional Whole-cell Patch Clamp Electrophysiology.
The Kv2.1 channel is a delayed rectifying, non-inactivating voltage-gated potassium channel that is widely expressed in the brain (Trimmer, 1991; Drewe et al., 1992; Gutman et al., 2005), in the heart and in muscle fibres (Gutman et al., 2005). It has been suggested to be critical for maintaining cell membrane potentials and for controlling the excitability of neurons (Misonou et al., 2005).

Current/Voltage Relationship:
The biophysical properties of the hKv2.1 channel were studied by stepping from the holding potential of the cell (-80 mV) to voltages of -60 mV to +60 mV in 10 mV increments every 10 s. The duration of each step was 200 ms (Figure 1). The hKv2.1 channel displays outward currents with very little inactivation (Figure 1A). These currents activated at membrane potentials between -20 and -30 mV (Figure 1B). This is in agreement with previously published findings (Shi et al., 1994; Wible et al., 1997; Coetzee et al., 1999).

Figure 1. A. Typical hKv2.1 current traces (upper panel) elicited by depolarizing voltage pulses from -60 mV to +60 mV in 10 mV increments (lower panel) from a holding potential of -80 mV. Scale bars represent 10 ms and 2 nA (upper panel) and 10 ms and 20 mV (lower panel) respectively.
B. I/V relationship of the hKv2.1 channel. Current amplitudes measured at the end of the 200 ms step. Currents normalized to the current amplitude obtained at +60 mV. The mean current at 60 mV was 5.63 ± 0.86 nA (n=11).

The membrane potential for half-activation ($V_{1/2}$) of the channel was found to be 4.9 ± 1.1 mV (mean ± SEM, n = 11 (Figure 2)). This is within the range of values, 2 - 12 mV, previously found for the Kv2.1 channel (Albrecht et al., 1993; Shi et al., 1994; Murakoshi et al., 1997; Wible et al., 1997; Coetzee et al., 1999; Gutman et al., 2005). The conductance-voltage plot had a slope value (k) of 12.1 ± 0.9 mV (Figure 2). Again, this value is within the range of published values (5 – 19 mV - Shi et al., 1994; Murakoshi et al., 1997; Wible et al., 1997; Coetzee et al., 1999).

**Figure 2. Normalized activation curve of the Kv2.1.**
Current (I) converted into conductance (G) by use of the following equation: $G = \frac{I}{V-E_K}$, where the Nernst $E_K$ was calculated as -89.82 mV. Data was fitted with a single Boltzmann equation. $V_{1/2} = 4.9 ± 1.1$ mV and slope (k) = 12.1 ± 0.9 mV. Values represent means ± SEM n = 11.
Pharmacology - 4-Aminopyridine:
The hKv2.1 channel was reversibly inhibited by 4-aminopyridine (4-AP). Increasing concentrations of 4-AP produced increasing inhibition of the depolarization-induced current (Figure 3A and 3B). The concentration of 4-AP that would reduce the current produced by 50% (IC$_{50}$) is between 1 and 10 mM (Table 1). This is in agreement with published findings, which give the IC$_{50}$ of 4-AP at the Kv2.1 channel as 0.5–3 mM (Shi et al., 1994; Coetzee et al., 1999).

Figure 3. The effect of 4-AP on the hKv2.1 current.
A. hKv2.1 current traces evoked by stepping to –20 mV from a holding potential of –80 mV in the presence of various concentrations of 4-AP. Upper panel; before the addition of 4-AP (Control - black trace), after addition of 1 mM 4-AP (light blue), 10 mM 4-AP (blue), 20 mM 4-AP (dark blue) and after wash off of 4-AP (grey). Scale bars represent 200 ms and 200 pA. Lower panel; voltage-step protocol. Scale bars represent 200 ms and 30 mV.

B. Mean hKv2.1 currents evoked by stepping to –20 mV from –80 mV. Currents normalized to control current. hKv2.1 channels were stepped for 1 s every 12 s.

Table 1. Percentage inhibition of the control current remaining after 3 minutes of application of different concentrations of 4-AP (n = 3).

<table>
<thead>
<tr>
<th>Concentration of 4-AP (mM)</th>
<th>Percentage inhibition of control current (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.8 ± 1.5</td>
</tr>
<tr>
<td>10</td>
<td>73.9 ± 1.7</td>
</tr>
<tr>
<td>20</td>
<td>81.2 ± 1.4</td>
</tr>
</tbody>
</table>
Pharmacology - Tetraethylammonium:
The hKv2.1 channel was reversibly inhibited by tetraethylammonium (TEA). Increasing concentrations of TEA produced increased inhibition of the current produced by depolarisation (Figure 4A and 4B). The IC$_{50}$ of TEA was found to be between 1 and 5 mM (Table 2). This is in agreement with the published values of 3.6 – 10 mM (Shi et al., 1994; Wible et al., 1997; Coetzee et al., 1999).

Figure 4. The effect of TEA on the hKv2.1 current.

A. hKv2.1 current traces evoked by stepping to 0 mV from a holding potential of –80 mV in the presence of various concentrations of TEA. Upper panel: before the addition of TEA (Control - black trace), after addition of 1 mM TEA (light green), 5 mM TEA (green), 10 mM TEA (dark green) and after wash off of TEA (grey). Scale bars represent 200 ms and 200 pA. Lower panel: Voltage-step protocol. Scale bars represent 200 ms and 30 mV.

B. Typical hKv2.1 currents produced by depolarizing pulse in the presence of various concentrations of TEA. hKv2.1 channels were stepped from a holding potential of –80 mV to 0 mV for 500 ms every 10 s.

Table 2. Percentage inhibition of the control current after application of TEA (n = 3-4).

<table>
<thead>
<tr>
<th>Concentration of TEA (mM)</th>
<th>Percentage inhibition of control current (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.6 ± 3.4</td>
</tr>
<tr>
<td>5</td>
<td>63.3 ± 1.1</td>
</tr>
<tr>
<td>10</td>
<td>76.0 ± 0.1</td>
</tr>
</tbody>
</table>
Stability of hKv2.1-CHO K1 Cell Line.

IonWorks™ HT Electrophysiology:

The hKv2.1-CHO K1 cell line has stable expression for >26 passages.

Functional channel expression, defined as cells expressing hKv2.1 current of ≥ 500 pA, was monitored using IonWorks™ HT. This data and the mean current amplitude is shown in Figure 5.

**Figure 5. Stability of expression over passage.** The upper panel shows the percentage of cells expressing a mean peak outward current >500 pA for cell passages 15, 19 and 26. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers adjacent red circles).
**Poly linker**: CMV-BamHI-NotI-**hKv2.1**-AscI-Clal—Hpal-EcoRI-IRES-neo

**hKv2.1 Sequence**:
(Accession Number NM_004975)
REFERENCES


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