PRODUCT DATASHEET

PrecisIOn™ hKv3.2-CHO Recombinant Cell Line

Catalog Number: CYL3044

PRODUCT DESCRIPTION
Recombinant CHO-K1 cell line expressing the human voltage-gated potassium channel Kv3.2.

ASSOCIATED PRODUCTS
The PrecisIOn™ hKv3.2-CHO Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:

- CYL3044SS  PrecisIOn™ hKv3.2-CHO Single Site License
- CYL3044TS  PrecisIOn™ hKv3.2-CHO Two Site License
- CYL3044MS  PrecisIOn™ hKv3.2-CHO Multiple Site License

CONTENTS
2 x 1 mL aliquots containing 2.64 x 10^6 cells/mL in 7.5% DMSO at passage 7.

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 degli 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 hKv3.2 were characterised in terms of their pharmacological and biophysical properties using whole-cell patch clamp techniques and IonWorks™ HT. Using whole-cell patch clamp techniques the threshold of current activation was found to occur when the membrane potential was depolarised to potentials more positive than -30 mV. The mean current at +40 mV was 9.55 ± 4.10 nA (n=7). Currents were inhibited by the known potassium channel blockers TEA and 4-AP in sub and low mM concentrations. The currents obtained with IonWorks™ HT were
typical of homomeric hKv3.2 currents since they had an initial fast transient component followed by a sustained component. Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 30 passages. At passage 30, 88% of cells sealed with a resistance >50 MOhm. Of these, 100% expressed hKv3.2 currents >500 pA with a mean current amplitude of 6.80 ± 0.12 nA (n=169).

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

**FUNCTIONAL VALIDATION**
Electrophysiological Properties of the hKv3.2 Current.

**Conventional Whole-Cell Patch Clamp Electrophysiology.**

**Current/Voltage Relationship:**
The Kv3.2 channel is a very slow inactivating delayed rectifying voltage-gated potassium channel that is found mainly in the brain (Rudy & McBain, 2001; Gutman et al., 2005).

The biophysical properties of the hKv3.2 current were studied by stepping from the holding potential of the cell (-80 mV) to voltages of -70 mV to +40 mV in 10 mV increments every 10 s. The evoked outward currents showed very little inactivation (Figure 1A). Substantial currents were only apparent when the membrane potential was depolarised to potentials more positive than -30 mV as expected if the current was carried by hKv3.2 channels (Figure 1B). Interestingly, the currents recorded under these conditions do not show the initial fast inactivating phase ascribed to potassium accumulation as seen in the IonWorks™ HT experiments (Figure 2). This may be due to the larger bath volume and continuous perfusion used in the conventional patch experiments.

**Figure 1. Current/Voltage relationship.**

**A.** Typical current traces (upper panel) elicited by 200 ms depolarising voltage pulses from -70 mV to +40 mV in 10 mV increments (lower panel) from a holding potential of -80 mV. Scale bars represent 10 ms and 1 nA (upper panel) and 10 ms and 20 mV (lower panel) respectively.
B. Mean I/V relationship. Current amplitudes were measured at the end of the 200 ms step and plotted against the test voltage. Currents were normalized to the current amplitude obtained at +40 mV. The mean current at +40 mV was 9.55 ± 4.10 nA (n=7).

IonWorks™ HT Electrophysiology.

To activate the currents the membrane voltage was stepped from a holding potential of -80 mV to +60 mV for 500 ms before returning to the holding potential. A typical current evoked by this protocol is shown in Figure 2. This current trace shows all the hallmarks of homomeric hKv3.2 currents since it has an initial fast transient component, thought to be due to local potassium accumulation resulting from fast activation (Lewis et al., 2004), followed by a sustained component, indicative of negligible inactivation over the duration of the 500 ms test pulse (Hernandez-Pineda et al., 1999).

Figure 2. A typical evoked current on IonWorks™ HT. A typical current (green trace) evoked by a voltage step to +60 mV (red trace).
Manual Patch Clamp Electrophysiology.

Pharmacology – 4-Aminopyridine:
The cell line evoked currents that were dose-dependently inhibited by 4-Aminopyridine (4-AP), an effect that was rapidly reversible (Figure 3). The mean dose-response data is shown in Table 1. The cell line is clearly sensitive to sub-mM concentrations of 4-AP as expected for the selective expression of hKv3.2.

Figure 3. The effect of 4-AP on hKv3.2 currents.
A. Current traces evoked by stepping to –10 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 0.5 mM 4-AP (green), 1 mM 4-AP (blue) and 5 mM 4-AP (red) respectively. Scale bars represent 100 ms (x-axis) and 500 pA (y-axis). Lower panel: voltage-step protocol. Scale bars represent 100 ms (x-axis) and 35 mV (y-axis).

B. Mean currents evoked by stepping to –10 mV for 1 s from –80 mV. Currents normalized to control current. hKv3.2 channels were stepped for 1 s every 12 s.

Table 2. Mean TEA dose-response data. Percentage of control current remaining after application of different concentrations of TEA (n=3).

<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>50.1 ± 2.1</td>
</tr>
<tr>
<td>5</td>
<td>79.0 ± 2.9</td>
</tr>
<tr>
<td>10</td>
<td>83.6 ± 2.5</td>
</tr>
</tbody>
</table>
IonWorks™ HT Electrophysiology.

Pharmacology – 4-AP and TEA:
The effect of 4-AP (Figure 5) and TEA (Figure 6) was tested using a 4 s voltage test step to +60 mV. The hKv3.2-CHO K1 cell line was sensitive to low mM concentrations of both 4-AP (IC\textsubscript{50} values were 6 mM and 3 mM for 4-AP and TEA respectively). The data generated on IonWorks™ HT is in agreement with previous literature reports. The Kv3.2 channel is reported to be highly sensitive to both 4-AP and TEA with concentrations of less than 1 mM reducing the current produced by approximately 50% (Coetzee \textit{et al.}, 1999; Hernandez-Pineda \textit{et al.}, 1999; Lien \textit{et al.}, 2002).

Figure 5. The effect of 4-AP on hKv3.2 currents.

Figure 6. The effect of TEA on hKv3.2 currents.
Stability of hKv3.2-CHO K1 Cell Line.

IonWorks™ HT Electrophysiology.

The hKv3.2-CHO K1 cell line has stable expression for > 30 passages.

Functional channel expression, defined as cells expressing hKv3.2 current of ≥ 500 pA, was monitored using IonWorks™ HT. This data and the mean current amplitude is shown in Figure 7. Number of cells expressing a current ≥ 500 pA shown above mean current amplitude data. Sealing data is shown in Figure 8.

Figure 7. Stability of expression over passage.
The upper panel shows the percentage of cells expressing a mean peak tail current >500 pA at cell passages 1, 6, 19, 24, and 30. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles).

Figure 8. Sealing rates over passage.
The percentage of cells sealing (defined as a seal resistance of >50 MΩhm).
Vector:

Polylinker: CMV-BamHI-NotI-Aval-Xhol-Ascl-hKv1.5-Hpal-EcoRI-IRES-neo

**hKv1.5 Sequence:**
The sequence of the cDNA used to make this cell line contains one silent mutation with respect to the accession number (NM_139136) **ACAA-ACG** (Thr) - at position 1535.
REFERENCES


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