human Kv1.3 Voltage-Gated K^+ Channel Cell Line

Product No.: AX-010-C

Lot No.: 1853762

Material Provided

Cells:	2 x 1 mL frozen aliquots (AX-010-CV)
Format:	~2.5 x 10^6 cells / mL in freezing medium

Product Information

Cellular Background:	CHO-DUKX
Cell Line Development:	CHO-DUKX cells were transfected using the pcDNA3.1(+) expression vector containing the coding sequence of the human Kv1.3 voltage-gated K^+ channel. Geneticin-resistant cells were selected and clones were obtained by limiting dilution and compared for their response to KCl in a membrane potential assay.
DNA Sequence:	Identical to coding sequence of GenBank NM_002232.2
Shipping Conditions:	Shipped on dry ice. Please ensure dry ice is still present in the package upon receipt or contact customer support.
Storage Conditions:	Store in liquid nitrogen (vapor phase) immediately upon receipt.

Quality Control

EC_{50} for a reference agonist is determined using a membrane potential assay. Mycoplasma test is performed using MycoAlert® Mycoplasma detection kit. *We certify that these results meet our quality release criteria.

KCl (EC_{50}):	N/D
Stability:	Cells were kept in continuous culture for at least 60 days and showed no drift in membrane potential assay response (EC_{50}, Emax).
Mycoplasma:	The cell line tested negative for Mycoplasma.

Recommended Cell Culture Conditions

Growth Medium: MEM Alpha (with L-glutamine) + 10% fetal bovine serum (FBS) + 0.4 mg/mL Geneticin (ion channel expression selection).

Freezing Medium: MEM Alpha (with L-glutamine) + 20% FBS + 10% DMSO.

Thawing Cells: Using appropriate personal protective equipment, place the frozen ampoule in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray ampoule with 70% ethanol and wipe excess with sterile towel. Under aseptic conditions using a pipette, transfer content to 10 mL complete medium and centrifuge (150 x *g*, 5 min). Resuspend cell pellet in 10 mL of complete medium and transfer to an appropriate culture flask. Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO_2.

Recommended Seeding Density: 11,000 – 15,000 cells/cm^2

Cell Culture Protocol: Cells are grown to 80% confluence, trypsinized (0.05% trypsin) and plated at 0.8-1.2 x10^6 cells in T75 flasks. Under these conditions, cell passages should be carried out every 3-5 days.

Typical Product Data – Membrane Potential Assay

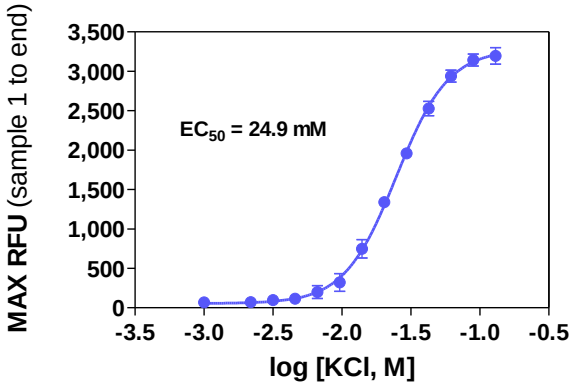
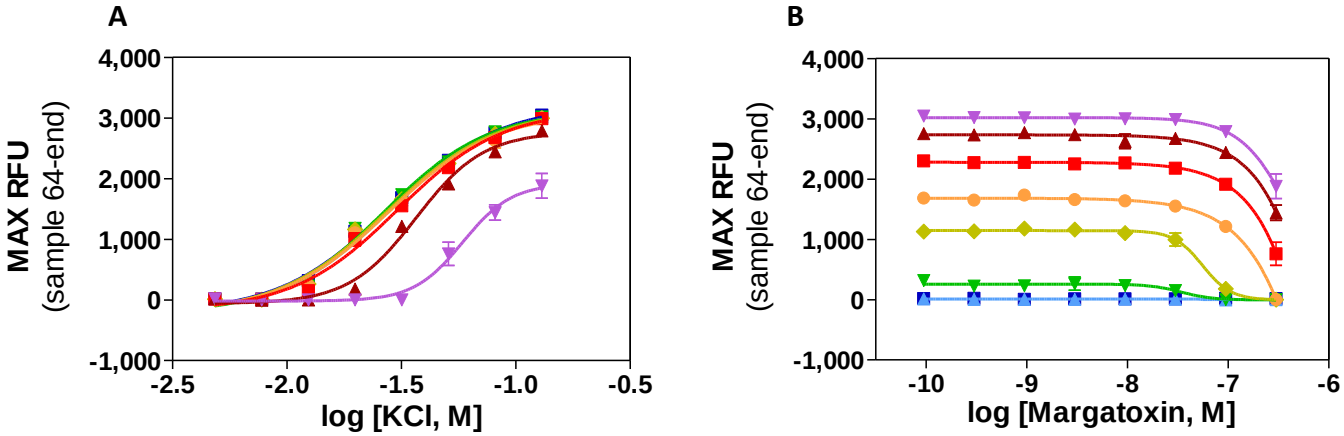


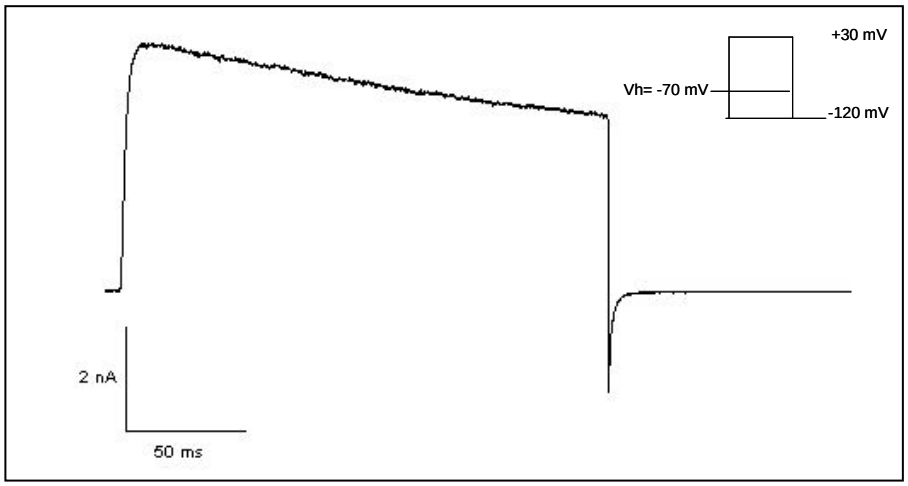
Figure 1: Agonist dose-response curve in a membrane potential assay.
10,000 cells/well were plated in a 384-well plate. Cells were stimulated in parallel with increasing concentrations of KCl in a membrane potential assay. Signal was detected with a FLIPR$^{TETRA®}$.



[Margatoxin]	EC_{50} (M)
Buffer	0.02786
0.3nM	0.02802
1nM	0.02663
3nM	0.02747
10nM	0.02780
30nM	0.03041
100nM	0.03673
300nM	0.05861

[KCl]	IC_{50} (M)
Buffer	
7.74 mM	5.389e-008
12.4 mM	3.418e-008
19.8 mM	5.558e-008
31.73 mM	~ 3.157e-006
50.78 mM	~ 7.592e-006
81.25 mM	~ 4.135e-006
130mM	~ 5.283e-007

Figure 2: Antagonist dose-response in a membrane potential assay.
10,000 cells/well were plated in a 384-well plate. Cells were pre-incubated for 5 min with the channel blocker margatoxin at the indicated concentrations before stimulation with KCl. The data were plotted in two different ways: A) as a function of KCl concentrations or B) as a function of margatoxin concentrations. Signal was detected with a FLIPR$^{TETRA®}$.



Mean current density at +30 mV: 847 ± 134 pA/pF.

No voltage-gated potassium channel current (i.e. -9.2 ± 1.7 pA/pF) was detected using the same protocol with the parental cell line

Figure 3: Voltage-dependent potassium current in whole cell voltage clamp configuration
Cells initially held at -70 mV were depolarized by 200 ms pulses up to + 30 mV. A typical delayed rectifier potassium current was induced in all the cells tested.

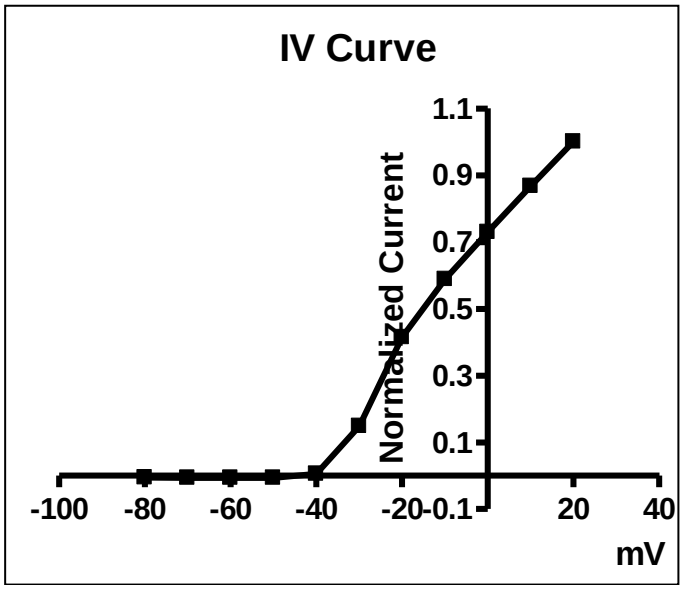


Figure 4: Voltage dependence of Kv1.3 activation - Whole cell voltage clamp
Cells were held at -70 mV. Depolarization pulses of 200 ms ranging from -80 mV to +20 mV were applied in 10 mV incremental steps. The steady state current at the end of the depolarizing pulses was plotted as a function of the voltage.

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Pharmacology Assay Procedure – Membrane Potential Assay

Materials:

Tyrode buffer: 130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM $NaHCO_3$, 20 mM HEPES; pH 7.4; sterile filtered and autoclaved.
Membrane Potential Assay Kit, Blue: The dye is diluted in Tyrode buffer as 2x stock (i.e. 1 bottle resuspended in 50 mL Tyrode), aliquoted and stored at -20°C.
0.625x Membrane Potential Blue dye solution: mix 5 mL of 2x dye + 11 mL of Tyrode buffer + 300 µl of probenicid solution (i.e. 71 mg probenicid dissolved in 500 µL NaOH 1N then further diluted to 1 mL by addition of 500 µL Tyrode buffer).

Methods:

1. Cells are plated at a density of 10,000 cells/well in 25 µL culture medium without antibiotic into black, clear bottom 384–well plates. Plates are incubated at 37°C for 24 h.
2. After medium removal, plates are incubated for 1 h at room temperature with 40 µL/well of 0.625x Membrane Potential Blue dye solution.
3. Signal is measured on the FLIPR$^{TETRA®}$ system equipped with a standard camera using the following protocol:
    - First injection: 10 µL/well of 5x compound solutions, prepared in Tyrode's buffer and containing 0.5% DMSO final concentration (injection parameters: 20 µL/s – 35 µL height).
    - Second injection: 25 µL/well of 3x Activator (KCl) solutions, prepared in Tyrode's buffer (injection parameters: 20 µL/s – 45 µL height).

Data Acquisition:
FLIPR$^{TETRA®}$ Read Interval was 5 s, with an Exposure Time of 0.5-0.8 s; Gain was set to 100 and Excitation Intensity to 40-100%.
Basal RFU values of each plate were adjusted to 1400-2000 RFU by varying the Gain or the Excitation Intensity values, then plates were injected with the corresponding compounds

The following Reading protocol was used for plate measurements:

- 15 s (3 samples; 5 s/sample) before the first injection
- 305 s (61samples; 5 s/sample) after the first injection
- 175 s (35 samples; 5 s/sample) after the second injection

FLIPR$^{TETRA®}$ measurements are analyzed with Screenworks© software (Molecular Devices, Version 2.0.0.24) and data are exported as Maximum (MAX) Statistics calculated from sample 64 (Start Reading Time of 2nd injection) to sample 99 (End Read=175 s after 2nd injection), after applying "Subtract Bias on Sample: 1" and "Spatial Uniformity" corrections.

Electrophysiology – Whole Cell Voltage Clamp

Intracellular solution:	128 mM $KMeSO_3$, 10 mM HEPES, 12 mM EGTA, 3 mM $MgCl_2$, 0.7 mM $CaCl_2$, 5 mM K_2ATP, pH 7.2 adjusted with KOH
Extracellular solution:	145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.4 adjusted with NaOH

Protocol:
For all the experiments, cells were held at -70 mV and currents were elicited by 200-ms depolarization pulses to +30 mV followed by a 50-ms pulse to –120 mV to measure tail currents. To obtain current-voltage relationships 200-ms voltage steps were applied in 10-mV increments to potentials that varied from -80 to +20 mV. The inter-pulse interval for all the protocols was 20s to allow full recovery from the inactivation process.

Data Acquisition:
Standard whole-cell voltage-clamp experiments were performed at room temperature. For data acquisition and further analysis, the EPC10 digitally controlled amplifier was used in combination with PATCHMASTER software (HEKA Electronics, Lambrecht, Germany). Capacitative currents were automatically subtracted by HEKA EPC10; currents were leak-subtracted using a P/4 protocol. The data were filtered at 3.33 KHz (-3dB, 4-pole Bessel lowpass) and digitized at 100 µs per point.

- Liquid junction potential: Corrected.
- Series resistance: The residual series resistances (after up to 92 % compensation) were 0.81 ± 0.17 MΩ (n=11).
- Pipette resistance and cell capacitance: The input resistance of the patch pipettes was 1.5-2.5 MΩ and the mean capacitance of the cells was 12.18 ± 0.43 pF (n=11).

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