

human TRPC3 Ion Channel Cell Line

Product No.: AX-011-C

Lot No.: 512-547-A

Material Provided

Cells:	2 x 1 mL frozen aliquot (AX-011-CV)
Format:	~2.5 x 10 ⁶ cells /mL in freezing medium

Product Information

Cellular Background:	HEK-293
Cell Line Development:	HEK293 cells were transfected using the pcDNA3.1(+) expression vector containing the coding sequence of the human TRPC3 ion channel. Geneticin-resistant cells were selected and clones were obtained by limiting dilution and compared for their response to carbachol in a membrane potential assay.
DNA Sequence:	Identical to coding sequence of GenBank NM_003305 with the exception of 2 synonym variations (a2274g and g2442a)
Receptor expression level (B _{max}):	Not determined for this cell line.
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

The EC₅₀ value for a reference agonist was determined using a membrane potential assay (Figure 1). A mycoplasma test was performed using MycoAlert[®] Mycoplasma (Lonza) detection kit. We certify that these results meet our quality release criteria.

Carbachol (EC ₅₀):	3100 nM
Stability:	Cells were kept in continuous culture for 20 passages (~ 60 days) and showed no drift in membrane potential assay response (EC ₅₀ , E _{max}).
Mycoplasma:	This cell line tested negative for Mycoplasma.

Recommended Cell Culture Conditions

Complete Medium: MEM/EBSS (with L-glutamine), 10% fetal bovine serum (FBS), 500 ug/mL Geneticin (ion channel expression selection).

Freezing Medium: MEM/EBSS(with L-glutamine), 10% fetal bovine serum (FBS) with 10% DMSO, without selection agents.

Thawing Cells: Using appropriate personal protective equipment, place the frozen aliquot in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray aliquot with 70% ethanol and wipe excess with a 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 (see recommended seeding density below). Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO₂.

Recommended Seeding Density: 41,000 – 45,000 cells/cm²

Cell Culture Protocol: Typically, for regular cell culture maintenance, these cells are grown to 80% confluence, trypsinized (0.05% trypsin) and plated at 2-4 x10⁶ cells in T75 flasks. Under these conditions, cell passages should be carried out every 3-5 days.

Typical Product Data - Membrane Potential Assay

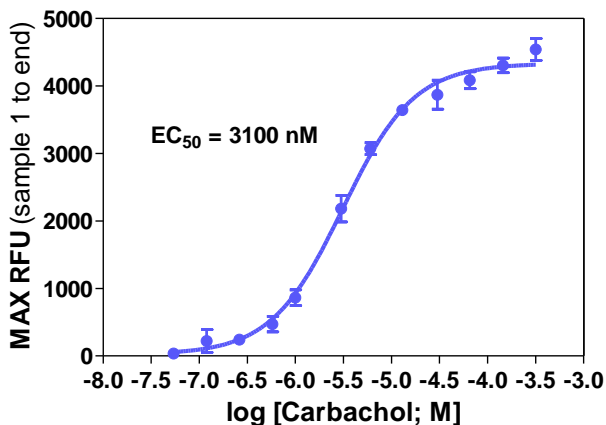


Figure 1: Agonist dose-response curve in a membrane potential assay. 15,000 cells/well were plated in a 384-well plate. Cells were treated in parallel with increasing concentrations of carbachol in a membrane potential assay. Carbachol activates an endogenous muscarinic receptor expressed in HEK cells, and the diacylglycerol formation and calcium store depletion that result from the muscarinic receptor activation are used to activate the TRPC3 channel. Signal was detected with a FLIPR^{TETRA}.

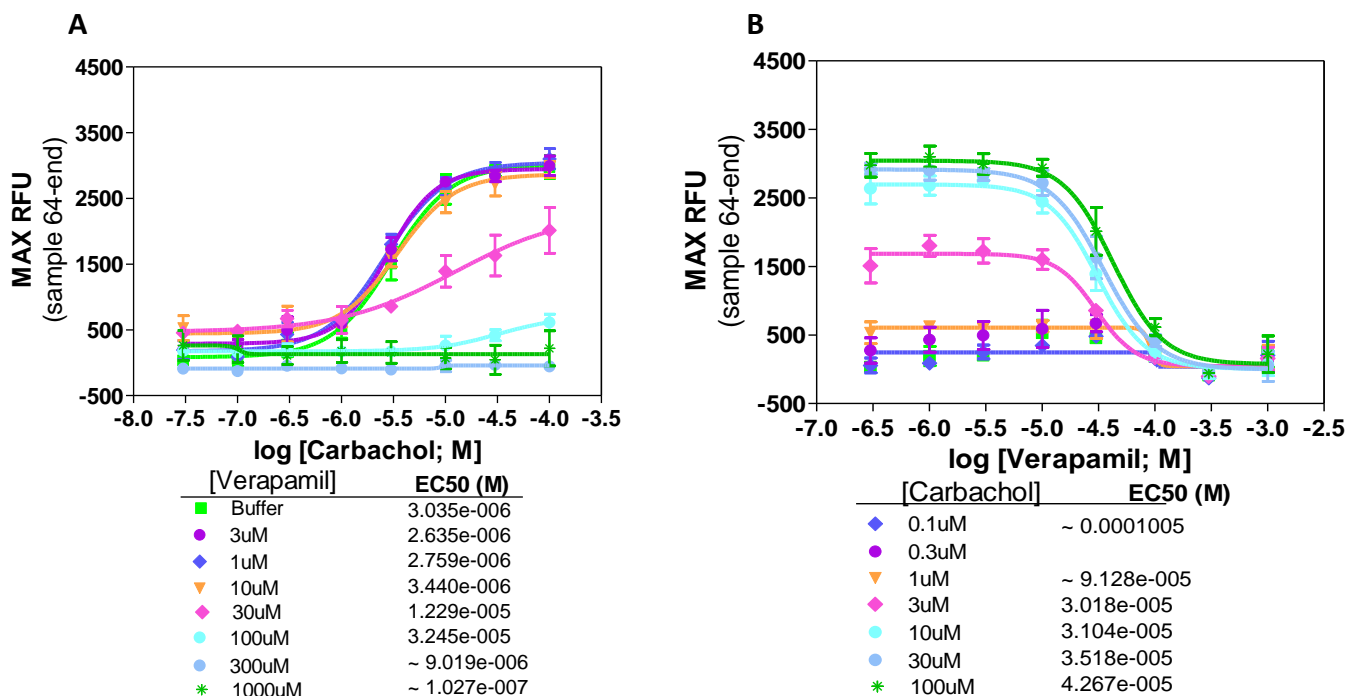
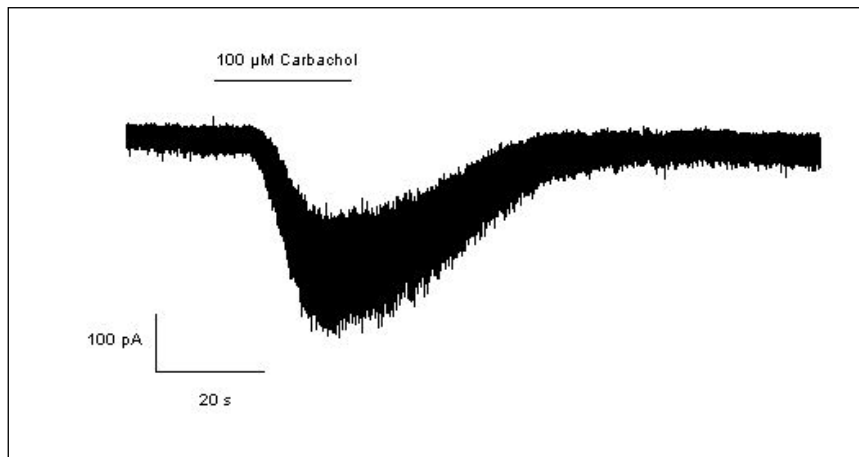


Figure 2: Antagonist dose-response in a membrane potential assay. 15,000 cells/well were plated in a 384-well plate. Cells were pre-incubated for 5 min with the channel blocker verapamil at the indicated concentrations before stimulation with carbachol. The data were plotted in two different ways: A) as a function of carbachol concentrations or B) as a function of verapamil concentrations. Signal was detected with a FLIPR^{TETRA}.

Typical Product Data - Electrophysiology



Mean current density at -80 mV:

-9.93 ± 1.74 pA/pF

Using the same protocol, the parental cell line showed no TRPC-like current

Figure 3: Activation of TRPC3 by 100 μM Carbachol in TRPC3 cells held at -80 mV. Application of 100 μM Carbachol in a whole cell voltage clamp protocol resulted in a slow activation\deactivation kinetics (Figure 3) and in a progressive desensitization (not shown).

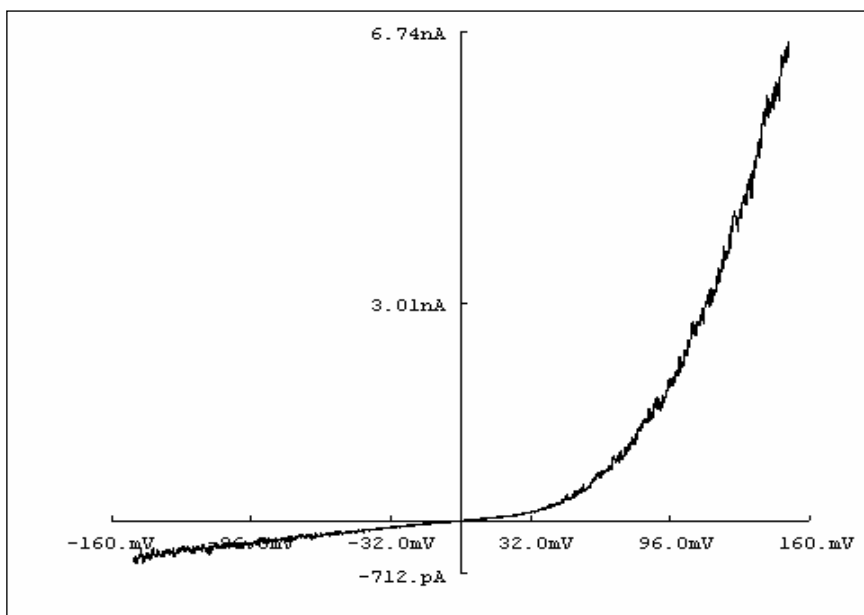


Figure 4: TRPC3 current-voltage relationship

A ramp protocol from -150 mV to +150 mV was applied for 300 ms before and after the application of 100 μM Carbachol in HEK-293 / TRPC3 cells. The leak current recorded before addition of carbachol was subtracted from the current recorded after addition of carbachol to generate the data shown here.



Pharmacology Assay Procedure - Membrane Potential Assay

Materials:

Low Calcium Tyrode buffer: 130 mM NaCl, 5 mM KCl, 0.15 mM CaCl₂, 1 mM MgCl₂, 5 mM NaHCO₃, 20 mM HEPES; pH 7.4; Sterile filtered and autoclaved.

Membrane Potential Assay Kit, Blue: The dye is diluted in Low Calcium Tyrode buffer as 2x stock, (i.e. 1 bottle resuspended in 50 mL Low Calcium Tyrode), aliquoted and stored at -20°C.

0.625x Membrane Potential Blue dye solution: mix 5 mL of 2x dye + 11 mL of Low Calcium Tyrode buffer + 160 µl of freshly prepared 3 mM BAPTA-AM (final BAPTA concentration if 30 µM)

Methods:

1. Cells are plated at a density of 15 000 cells/well in 25 µL culture medium without antibiotic into black, clear bottom Poly-D-Lysine coated 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 Low Calcium 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 (carbachol) solutions, prepared in Low Calcium 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 0.8 s; Gain was set to 60-120 and Excitation Intensity to 70-100%.

Basal RFU values of each plate were adjusted to 1300-1500 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
- 300 s (61 samples; 5 s/sample) after the first injection
- 300 s (59 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 123 (End Read=300 s after 2nd injection), after applying "Subtract Bias on Sample: 1" and "Spatial Uniformity" corrections.



Electrophysiology – Whole Cell Voltage Clamp

Intracellular solution: 128 mM CsCl, 12 mM EGTA, 3 mM MgCl₂, 0.7 mM CaCl₂, 10 mM HEPES, 5 mM Na₂ATP, pH 7.2 with CsOH.

Extracellular solution: 145 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM HEPES, 10 mM Glucose, pH 7.4 with NaOH.

Protocol:

For all the experiments, cells were held at -80 mV and currents were elicited by addition of extracellular carbachol in continuous recording mode. Voltage ramps (holding potential = 0 mV) from -100 mV to +100 mV over 300 ms were applied before and after the application of carbachol.

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 by mean of prepulse. The data were filtered at 3.33 KHz (-3dB, 4-pole Bessel lowpass) and digitized at 100 μ s per point.

- Liquid junction potential: no correction.
- Series resistance: The residual series resistances (after up to 80 % compensation) were 2.36 ± 0.32 M Ω (n=10).
- Pipette resistance and cell capacitance: The input resistance of the patch pipettes was 2-4 M Ω and the mean capacitance of the cells was 14.76 ± 1.39 pF (n=10).

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