B'SYS GmbH

CHO TRPV1 Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The TRPV1 channel

The transient receptor potential TRPV1 vanilloid receptor gates a non selective cation channel that is expressed by sensory neurons and that can be activated by protons, heat and capsaicin (Seabrook et al., 2002).

1.2 B'SYS' CHO TRPV1 Cells

B'SYS has designed a new CHO TRPV1 cell line with constitutive expression of human TRPV1 channel. The human TRPV1 cDNA was cloned and transfected into CHO cells and then the functional properties of the TRPV1 channel were validated by means of the patch-clamp technique in manual patch clamp and on the QPatch automate. The validation was made with 3 stimuli: either using a pH5 bath solution, a 45°C heated bath solution or the TRPV1 agonist capsaicin. The TRPV1 antagonist capsazepine was also used to block the channel at different concentrations depending on the stimulus used to activate the channel. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human TRPV1 channel:

- 1 x 0.5 mL aliquots of frozen cells at 2.4 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO

2.2 Mycoplasma Certificate

B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

3 VALIDATION OF CHO TRPV1 CELLS

3.1 Methods

TRPV1 currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1, $MgCl_2$ 1, Hepes (or MOPS for pH 5) 10, D-Glucose 10. The pH was adjusted to 7.4 with NaOH. The pipette solution consisted of (in mM) CsCl 120, TEA-Cl 20, Na-ATP 1.2, Na-GTP 0.2, Hepes 10, EGTA 10. The pH was adjusted to 7.20 with KOH. After formation of a GΩ seal between the patch electrodes and individual TRPV1 stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established the cells were clamped at -60 mV. The TRPV1 currents were activated by the stimuli Capsaicin, low pH or high temperature. The corresponding EC_{50} values were determined. To evaluate the outward TRPV1 current and to control the quality of the seal a ramp starting at -60 mV to +60 mV was applied every 10 s (duration 0.5 s) in the manual patch clamp assay. TRPV1 currents were blocked by the agonist Capsazepine to control cell sensitivity.

3.2 Manual Patch Clamp

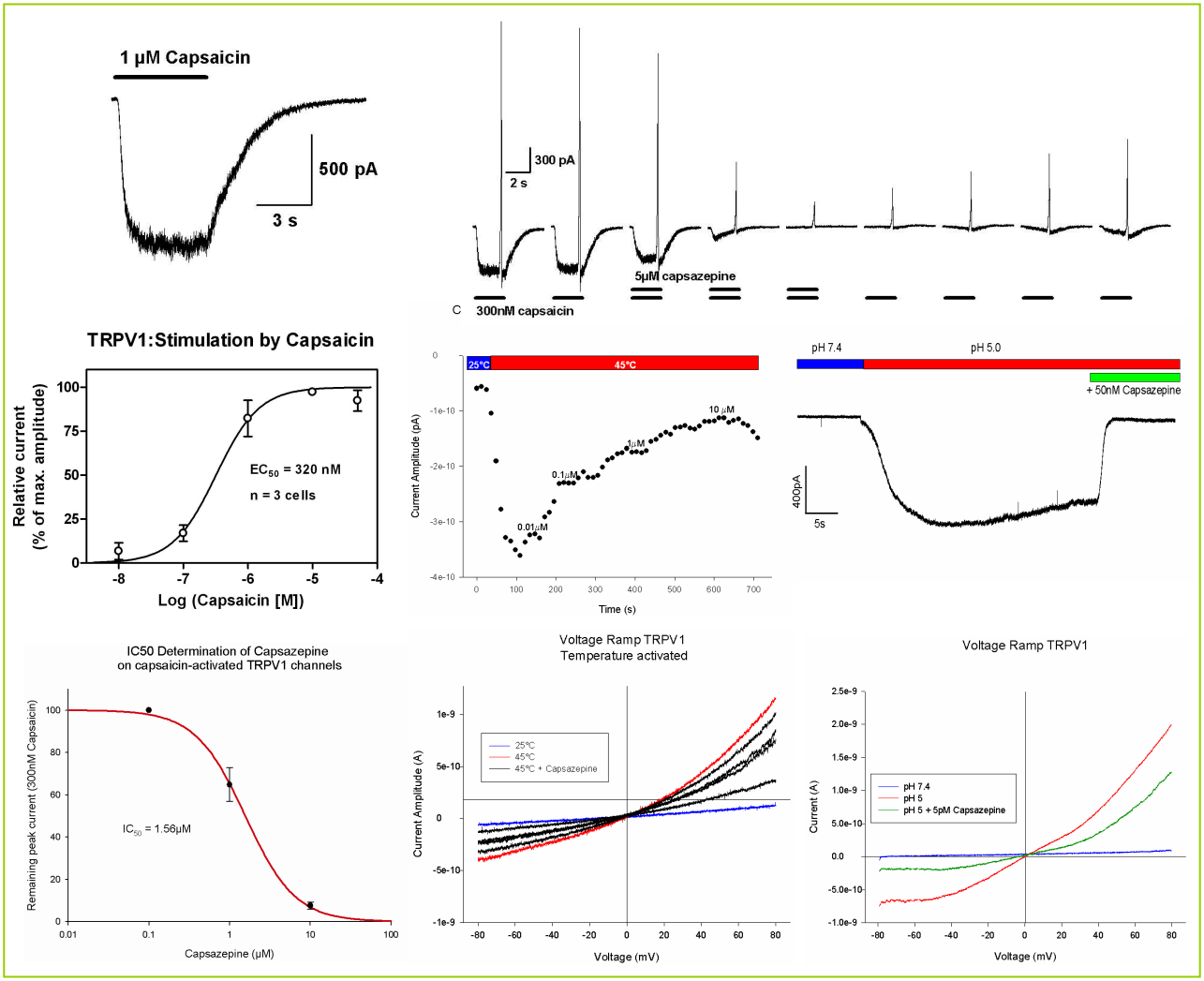

Fig.1: From top to bottom and from left to right: Activation of TRPV1 current by 1 µM capsaicin / Block of capsaicin-activated (300 nM) TRPV1 steady-state and ramp currents by 5 µM capsazepine and partial wash-out / Dose-response curve of capsaicin-activated TRPV1 currents / Block of temperature-activated TRPV1 current by different concentrations of capsazepine / Block of pH-activated TRPV1 by 50 nM capsazepine / Capsazepine IC_{50} determination on capsaicin-activated TRPV1 channels / Voltage ramps of heat-activated TRPV1 / Voltage ramps of pH-activated TRPV1 channels.

3.3 Automated Patch Clamp

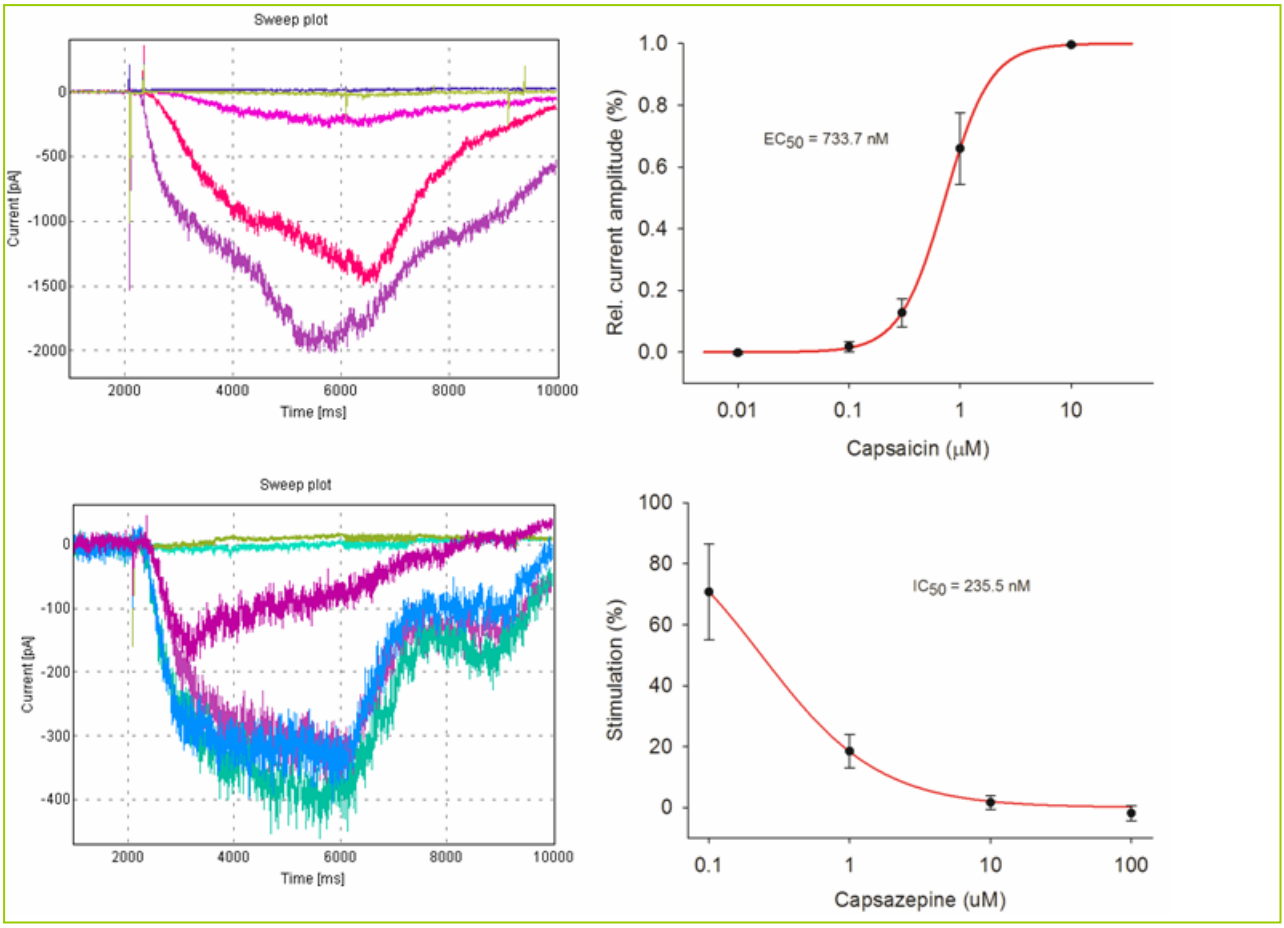

Fig.2: From top to bottom and from left to right: Activation of TRPV1 currents by increasing concentrations of capsaicin (0.1, 1, 10, 100 µM) / Dose-response curve of capsaicin-activated TRPV1 currents (EC_{50}: 733.7 nM) / Block of capsaicin-activated (1 µM) TRPV1 currents by increasing concentrations of capsazepine (0.01, 0.1, 0.3, 1, 10 and 30 µM) / Capsazepine IC_{50} determination on capsaicin-activated TRPV1 channels (IC_{50}: 235.5 nM)

3.4 Fluorescence based assays

The Calcium influx in CHO TRPV1 cells was analyzed using a FLEXstation in combination of the Calcium Plus Assay Kit (Molecular Devices). 8 x 104 cells were seeded per well of a black 96 well plate with clear bottom. The assay was carried out in Hanks Balanced Salt solution (HBSS, calcium, magnesium, no phenol red) containing 2-2.5 mM Probenicid. Increasing capsaicin concentrations were added after recording the baseline fluorescence for 45 s. The time course of the fluorescence was recorded.

The experimental setup parameters:

Excitation wavelength: 584 nm
Emission wavelength: 525 nm
Emission cut-off: 515 nm

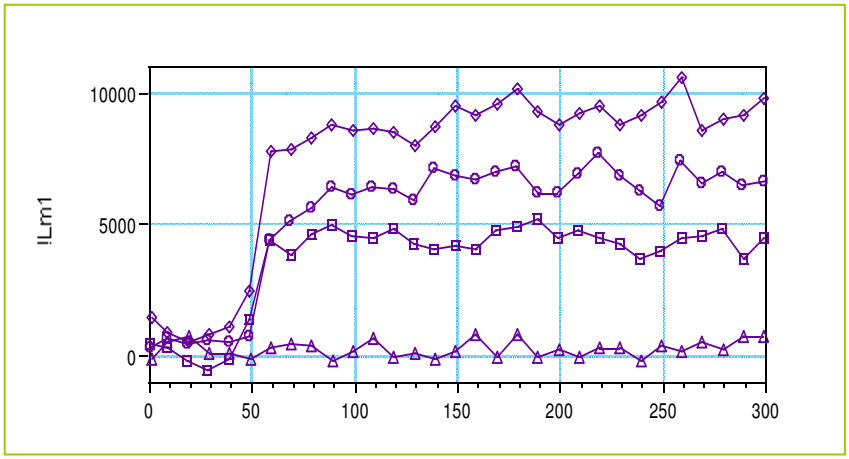

Fig. 3: Overlay of fluorescence signals recorded from wells treated with increasing concentrations of Capsaicin.

4 CELL CULTURE CONDITIONS

4.1 General

CHO TRPV1 cells are incubated at 37°C in a humidified atmosphere with 5% CO_2 (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing F12 (HAM) medium supplemented with 9% fetal bovine serum, 0.9% Penicillin/Streptomycin solution and 500 µg/mL G-418 suplhate. The CHO TRPV1 cells are passaged at a confluence of about 80%. For electrophysiological measurements the cells are seeded onto e.g. 35 mm sterile culture dishes containing complete medium.

- All solutions and equipment coming in contact with the cells must be sterile.
- Use proper sterile technique and work in a laminar flow hood.
- Be sure to have frozen cell stocks at hand before starting experiments.
- Cells should be split every 3-4 days at 70% - 80% confluency at 1:3 to 1:10 ratio.

4.2 Recommended Complete Medium

- F12 (HAM) with GlutaMAX I or L-Glutamine
- 11% FBS
- 1.1% Penicillin/Streptomycin

4.3 Antibiotics

- CHO TRPV1 clones were selected under 500 µg/mL G-418 sulphate antibiotic pressure.
- To cultivate CHO TRPV1 cells, also the same antibiotic pressure (100 µg/mL) can be used.
- To separate CHO TRPV1 cells from untransfected cells, use 500 µg/mL G-418 sulphate.

Remark: The permanent application of high antibiotic pressure has no effect on current density.

4.4 Thawing Cells

- Remove vial of cells from liquid nitrogen and thaw quickly at 37°C.
- Decontaminate outside of vial with 70% ethanol.
- Transfer cells to a T-75 culture flask containing 15mL complete medium.
- Incubate cells at 37°C for 4-6 h to allow the cells to attach to the bottom of flask.
- Next day aspirate off the medium and replace with 15 mL complete medium & antibiotics.
- Antibiotics: 500µg/mL G-418 sulphate.
- Incubate cells and check them daily until 70% - 80% confluency is reached.

4.1. Splitting Cells

- When cells are 70% - 80% confluent remove medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
- Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.
- Add 4 µM capsazepine blocker to the medium.

4.5 Freezing Medium

- Mix 0.9 mL fresh complete medium and 0.1 mL DMSO for every 1 mL freezing medium.
- Sterilize freezing medium by means of appropriate micro filter (0.1 µm – 0.2 µm).

4.6 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluency prior to freezing.
- Remove the complete medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
- Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells with centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.5 mL of cell suspension into each cryovial.
- Overnight incubate cells in a styropor box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4.7 Stability of CHO TRPV1 cells

CHO TRPV1 cells stably express functionally active TRPV1 channels over 35 passages. Under recommended cell culture conditions no variation in current density was observed over 35 cell splitting cycles.

5 TRPV1 SEQUENCE

5.1 Human TRPV1 Accession Number NM_080704.2

Cloned cDNA sequence of human TRPV1 subunit was error-free and identical with NM_080704.2 sequence:

```
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```

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