B'SYS

B'SYS GmbH

CHO TRPA1 Cell Line

Specification Sheet

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1 BACKGROUND

1.1 B'SYS' TRPA1 Cells

B'SYS has designed a CHO TRPA1 receptor cell line with constitutive expression of the receptor. The human TRPA1 receptor cDNA was cloned and transfected into CHO cells and then the functional properties of the TRPA1 receptors validated by means of manual patch-clamp recordings. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human TRPA1 receptors:

- 1.5 mL aliquots of frozen cells at approximately 1.5 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 TRPA1 CELLS

3.1 Electrophysiology

TRPA1-mediated currents were measured by means of the patch-clamp technique in the perforated patch configuration. The bath solution to obtain a GΩ seal contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. As soon as a GΩ seal was obtained, the cells were perfused with the following bath solution (in mM) NaCl 140, KCl 5, $MgCl_2$ 2, EGTA 5, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) K-Methanesulfonate 120, KOH 6, KCl 20, $MgCl_2$ 0.8, $CaCl_2$ 0.8, MgATP 4, HEPES 10, BAPTA 1, Amphotericin B 480 µg/mL, pH (Methanesulfonic acid) 7.20.

After formation of a GΩ seal between the patch electrodes and individual TRPA1 stably transfected CHO cells, voltage ramps from -80 to +80 mV (500 ms) were applied every 10 sec to elicit TRPA1-mediated currents. Pharmacological treatment was started once full perforation of the membrane was achieved, as monitored by the change of the series resistance (Rs). All solutions applied to cells were maintained at room temperature. The holding potential was -40 mV.

3.2 AITC as Agonist

TRPA1 channels were activated with concentrations of allyl isothiocyanate (AITC 0.3 µM – 100 µM), one concentration was applied per cell. The EC_{50} for AITC was calculated to be 14.12 µM (Hill coefficient 1.74).

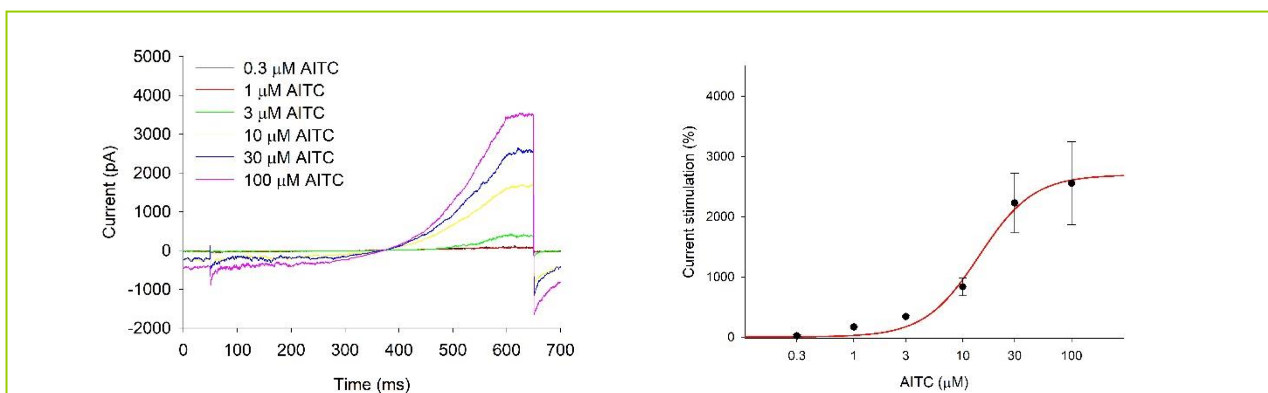

Fig.1: Representative current traces and concentration-response curve for AITC applied to TRPA1 cells. The current at +80mV was analysed for the generation of the curve (n=3-4). One concentration was applied per cell and current amplitude normalised to the baseline current before AITC addition.

3.3 Antagonist

As antagonist the selective TRPA1 channel blocker HC-030031 was tested at concentrations between 0.3 µM and 100 µM in the presence of 5.0 µM AITC. The IC_{50} was determined to be: 6.33 µM (Hill coefficient: 1.02) when currents were analysed at +80 mV. The IC_{50} for currents analysed at -80 mV was determined to be 3.16 µM (Hill coefficient: 0.95).

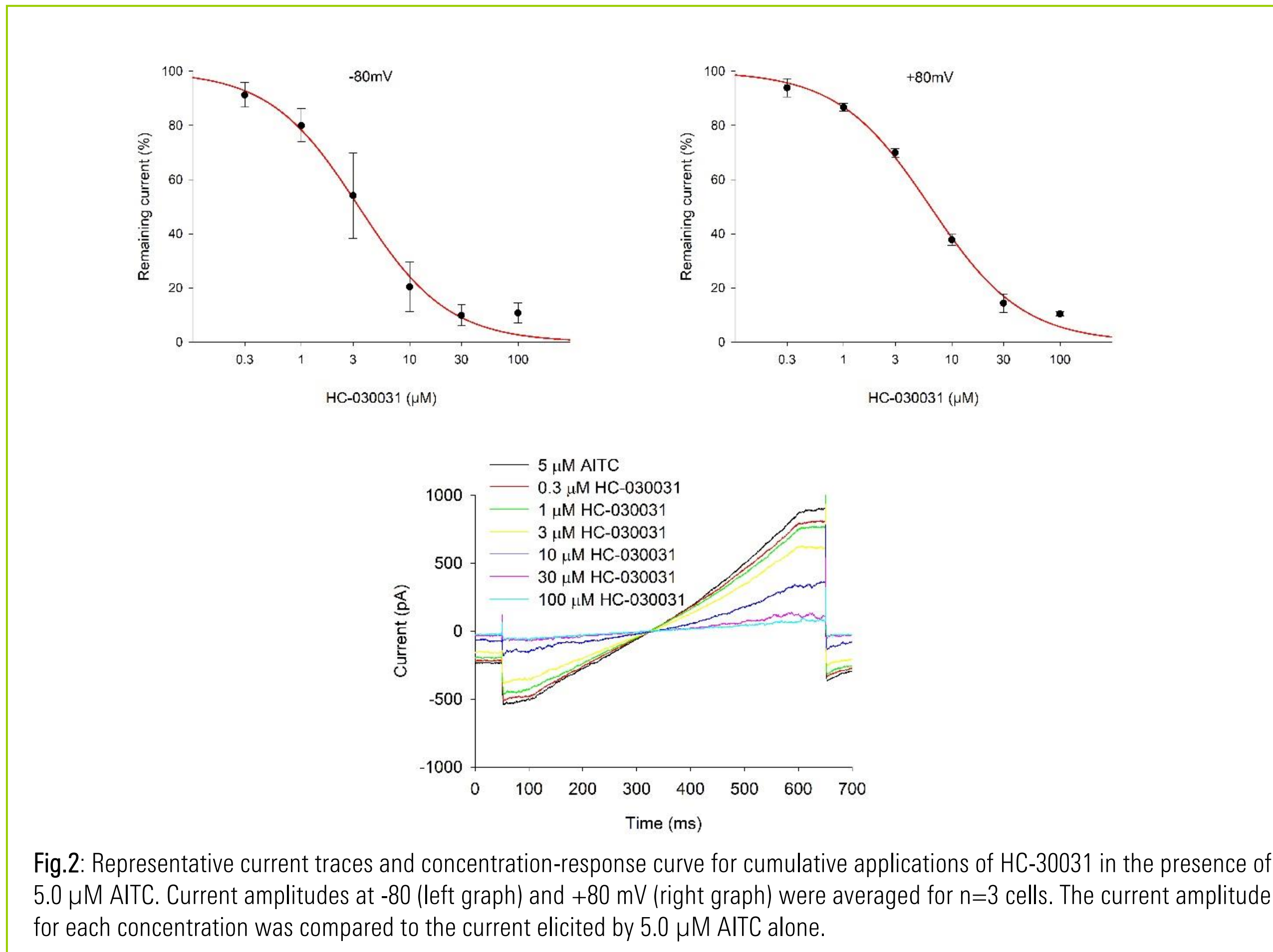

Fig.2: Representative current traces and concentration-response curve for cumulative applications of HC-30031 in the presence of 5.0 µM AITC. Current amplitudes at -80 (left graph) and +80 mV (right graph) were averaged for n=3 cells. The current amplitude for each concentration was compared to the current elicited by 5.0 µM AITC alone.

4 CELL CULTURE CONDITIONS

4.1 General

CHO TRPA1 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 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and Hygromycin 250 µg/mL. The CHO TRPA1 receptor cells are passaged at a confluence of about 50-80%. 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 2 to 3 days at 50% to 80% confluency at 1:3 to 1:5 ratio.

Table 1: Cell culture reagents

Product	Supplier	Order number
Nutrient mixture F-12 Ham (F12 Ham)	Sigma-Aldrich	N6658
Fetal Bovine Serine (FBS)	Gibco	10270-106
Penicillin / Streptomycin (100x)	Gibco	10378-016
Phosphate Buffered Saline (PBS, without Ca^{2+} and Mg^{2+})	Sigma-Aldrich	D8537
Hygromycin B (50 mg/mL)	Gibco	10687010
Detachin	Genlantis	T100T100
Trypsin EDTA (10x)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438

For the preparation of 1X Trypsin/EDTA, the 10X solution is diluted in PBS (without Ca^{2+} and Mg^{2+}), aliquoted and stored in the freezer.

4.2 Recommended Complete Medium

- DMEM/F12 with L-Glutamine or GlutaMAX I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- CHO TRPA1 receptor clones were selected under Hygromycin 400 µg/mL, antibiotic pressure.
- To cultivate TRPA1 receptor cells, a reduced antibiotic pressure (Hygromycin 250 µg/mL) should be used.

Remark: The permanent application of 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-25 culture flask containing 5 mL complete medium
- Incubate cells at 37°C for at least 4-6 hours to allow the cells to attach to the bottom of the flask

- Once cells attach to the bottom of the flask and look healthy, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see 4.3)
- To check whether cells are attached properly, the flask can be gently moved while looking under the microscope
- If 48h after thawing the confluency is below 50%, replace the medium in the flask with fresh medium containing antibiotics
- Incubate cells at 37°C and check them daily until 50% to 80% confluency is reached.

4.5 Splitting Cells

- When cells are 50% to 80% confluent remove complete medium.
- Wash cells with 1xPBS to remove excess medium
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C.
- Detach cells by gently tapping the sides of the flask 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.
- Use remaining suspension for counting the cells.

4.6 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).

4.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluency prior to freezing.
- Remove the complete medium and wash cells with 1xPBS.
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C
- Detach cells by gently tapping the sides of the flask, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells at 200 g using a centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 1.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.75 mL of cell suspension into each cryovial.
- Overnight incubate cells in a polystyrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

5 SEQUENCE

Cloned cDNA sequence of human TRPA1 subunit was error-free and encodes for NP_015628:

5.1 Human TRPA1 Accession Number NP_015628

```
MKRSLRKMWRPGEKKEPQGVVYEDVPDDTEDFKESLKVVFEGSAYGLQNFNKQKKLKRCDDMDTFFLHYAAAEGQIELME
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EMEILKQKYRLKDLTFLLEKQHELIKLIIQKMEIISETEDDDSHCSFQDRFKKEQMEQRNSRWNTVLRAVKAKTHHLEP
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6 CONTACT INFORMATION

6.1 Contact Address for Technical Support & Ordering Information

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