B'SYS GmbH

CHO ASIC1b Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The ASIC1 channel

Cation channel with high affinity for sodium, which is gated by extracellular protons and inhibited by the diuretic amiloride. Also permeable for Ca^{2+}, Li^{+} and K^{+}. Generates a biphasic current with a fast inactivating and a slow sustained phase. Mediates glutamate-independent Ca^{2+} entry into neurons upon acidosis. This Ca^{2+} overloading is toxic for cortical neurons and may be in part responsible for ischemic brain injury. Heteromeric channel assembly seems to modulate channel properties. Functions as a postsynaptic proton receptor that influences intracellular Ca^{2+} concentration and calmodulin-dependent protein kinase II phosphorylation and thereby the density of dendritic spines. Modulates activity in the circuits underlying innate fear.

1.2 B'SYS' CHO ASIC1b Cells

B'SYS has designed a new CHO ASIC1b cell line with constitutive coexpression of human Acid-sensing ion channel 1 (=Amiloride-sensitive cation channel 2). The human ASIC1b cDNA was cloned and transfected into CHO cells and then the functional properties of the ASIC1b channels validated by means of the patch-clamp technique. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human ASIC1b channel:

- 1 x 0.5 mL aliquots of frozen cells at 1.0 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 ASIC1B CELLS

3.1 Electrophysiology

ASIC1b 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, CaCl2 1.8, MgCl2 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, MgCl2 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual ASIC1b 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 desensitizing currents were measured upon low pH stimulation at a holding potential of -80 mV (Fig. 1). The cell was stimulated after a minimum rest of 30 s.

3.2 pH dependence

The cells were stimulated with solutions of increasing pH. The resulting pH_{50} was determined as 6.57 (n=6). This value is in good agreement with data from literature (Hesselager et al. 2004).

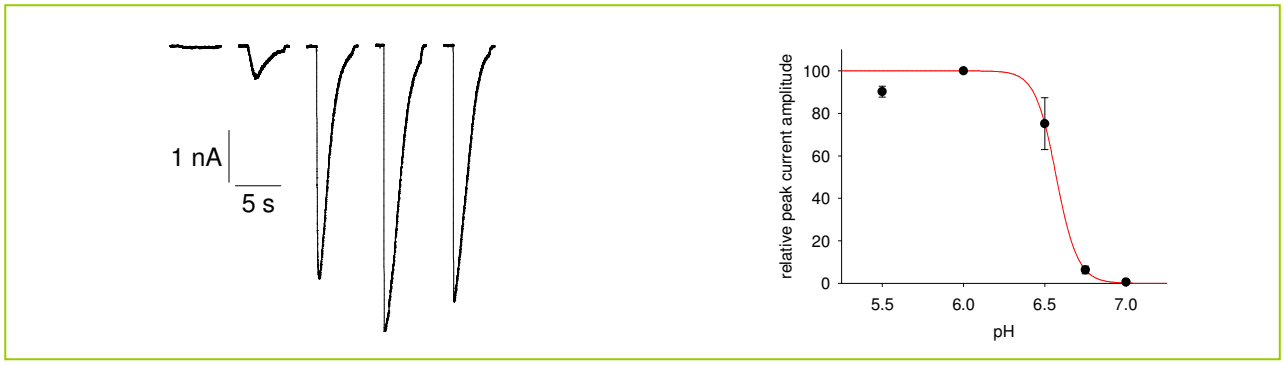

Fig.1: pH activation of ASIC1b currents upon depolarization of the cell membrane at a holding potential of -80 mV. left: currents recorded at pH 7.0, 6.75, 6.5, 6.0 and 5.5. Right: pH Dose response curve. The pH_{50} was determined to be 6.57 (n=6) No currents were recorded in untransfected cells (data not shown).

3.3 Block by Amiloride

Cells were stimulated with solutions at pH 6.5. Increasing concentrations of Amiloride were co-applied and the peak current amplitudes were plotted versus the Amiloride concentration. The dose response curve was generated and an IC_{50} of 18.14 µM was determined (Hill coefficient: 1.07)

Fig.2: Amiloride block of ASIC1b currents. Cells were stimulated with pH6.5. pH6.5 was applied twice followed by increasing concentrations of Amiloride: 30, 100, 300, 1000 µM dissolved in bath solution (pH 6.5)

4 CELL CULTURE CONDITIONS

4.1 General

CHO ASIC1b cells are incubated at 37°C in a humidified atmosphere with 5% CO2 (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 100 µg/mL Hygromycin. The CHO ASIC1b 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.
- Cells should be split every 2-3 days at 70% - 80% confluency at 1:3 to 1:5 ratio.

4.2 Recommended Complete Medium

- F12 (HAM) with L-Glutamine
- 9% FBS
- 0.9% Penicillin/Streptomycin

4.3 Antibiotics

- CHO ASIC1b clones were selected under 100 µg/mL Hygromycin antibiotic pressure.
- To separate CHO ASIC1b cells from untransfected cells, use 1000 µg/mL Hygromycin.

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-25 culture flask containing 5 mL complete medium.
- Incubate cells at 37°C for 4-6 h to allow the cells to attach to the bottom of flask.
- Aspirate off the medium and replace with 5 mL complete medium & antibiotics.
- Antibiotics: 100 µg/mL Hygromycin.
- Incubate cells and check them daily until 70% - 80% confluency is reached.

4.5 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.
- 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 (0.1µm – 0.2µm) or use sterile DMSO.

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.
- 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 1.0 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.

4.8 Stability of CHO ASIC1b cells

CHO ASIC1b cells stably express functionally active ASIC1b channels over 23 passages. Under recommended cell culture conditions no variation in current density was observed over 23 cell splitting cycles.

5 ASIC1B SEQUENCE

5.1 Human ASIC1b

Cloned cDNA sequence of human ASIC1b subunit was error-free. The cDNA encodes for the isoform 3 (isoform c) of Acid-sensing ion channel 1 (ASIC1b). Accession number: NP_001243759.1

MPIQIFCSMSFSSGEEAPGPLGDIWGPHHHQQQQDISESEEEEEEKEKEAVRKEASEGHSPMDLVAFANSCTLHGTNHIF
VEGGPGPRQVLWAVAFVLALGAFLCQVGDRVAYYLSYPHVTLLNEVATTELAFPAVTLCNTNAVRLSQLSYPDLLYLAPM
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CNLTRYGKELSMVKIPSKASAKYLAKKFNKSEQYIGENILVLDIFFEVLNYETIEQKKAYEIAGLLGDIGGQMGLFIGAS
ILTVLELFDYAYEVIKHKLCRRGKCQKEAKRSSADKGVALSLDDVKRHNPCESLRGHPAGMTYAANILPHHPARGTFEDF
TC*

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