B'SYS GmbH

CHO ASIC3 Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The ASIC3 Channel

Member of the degenerin/epithelial sodium channel superfamily (DEG/ENaC). Cation channel with high affinity for sodium (also permeable for Ca^{2+}, Li^{+} and K^{+}) gated by extracellular protons and inhibited by the diuretic Amiloride. It generates a biphasic current with a fast inactivating and a slow sustained phase and may play an important role in the detection of lasting pH changes. ASIC3 mediates glutamate-independent Ca^{2+} entry into neurons upon acidosis and therefore 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 ASIC3 Cells

B'SYS has designed a new CHO ASIC3 cell line with constitutive coexpression of human Acid-sensing ion channel 3 (=Amiloride-sensitive cation channel 3). The human ASIC3 cDNA was cloned and transfected into CHO cells and then the functional properties of the ASIC3 channels validated by means of the patch-clamp technique. The assays are fully validated for manual and automated patch-clamping (Q-Patch). Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human ASIC3 channel:

- 1 x 1 mL aliquots of frozen cells at 1.5 x 10^6 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 ASIC3 CELLS

3.1 Electrophysiology

ASIC1b currents were measured by means of the patch-clamp technique in the whole-cell configuration. The standard bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7 or 8. For stimulation of the channel bath solutions with lower pH were used. In this case HEPES was replaced by MES (pH6 and 6.5) or MOPS (pH5 and 5.5)

The pipette solutions had the following composition:

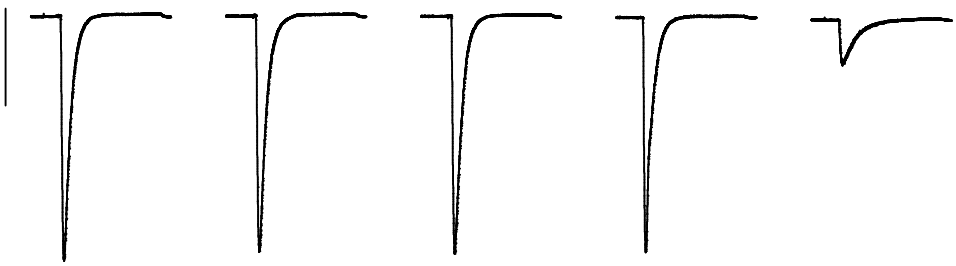
MANUAL PATCH CLAMPING		**AUTOMATED PATCH CLAMPING**	
Components	**Concentration (mM)**	**Components**	**Concentration (mM)**
Potassium Chloride	130	Potassium Chloride	130
Magnesium Chloride	1	Calcium Chloride	2
Magnesium ATP	5	Magnesium Chloride	4
HEPES	10	Sodium ATP	4
EGTA	5	HEPES	10
		EGTA	5

After formation of a GΩ seal between the patch electrodes and individual stably transfected CHO ASIC3 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.

3.2 PH Dependence

Manual Patch Clamping:

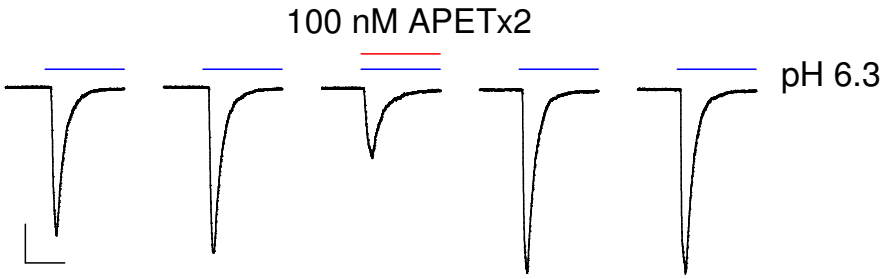
ASIC3 currents were stimulated by acidic bath solution (pH6.5). The antagonist Amiloride hydrochloride at a concentration of 100 µM was used to block the currents. Between two applications bath solution of pH8 was perfused for at least 30 s.



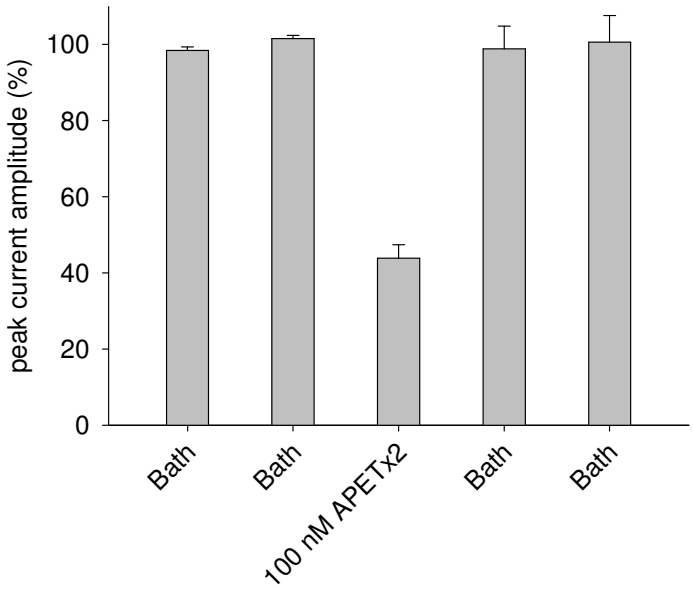
Acidic bath solution (pH 6.5) + 0, 0, 0, 0.5% vehicle (50% Ethanol / 50% Water (v/v)), 100 µM Amiloride hydrochloride

3.3 APETx2, a specific blocker of ASIC3

The ASIC3 specific blocker APETx2 was tested at one concentration (100 nM) using manual patch-clamping. The cells were perfused with a bath solution (pH8.0) and ASIC3 currents were stimulated by applying a pH6.3 bath solution. The inhibitor APETx2 was co-applied. The current amplitude decreased to 43.83 ± 3.56% (n=7) of the initial current amplitude. The effect was reversible. This result is in agreement with published values (Diochot S et al. 2004 and Karczewski J et al 2010).



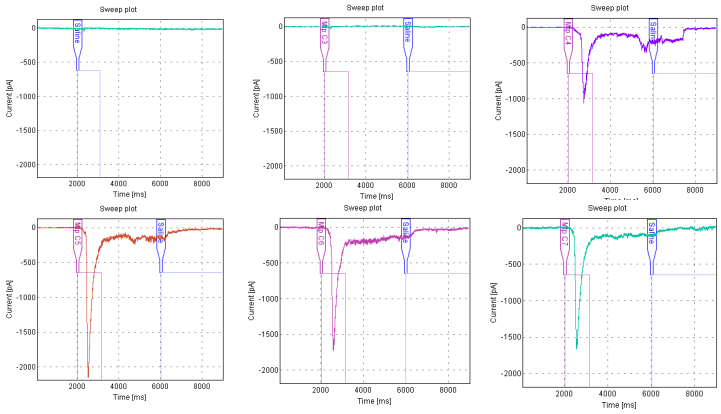
Effect of 100 nM APETx2: ACIC3 currents were stimulated by changing pH from 8.0 to 6.3. 100 nM APETx2 was coapplied and blocked 56.13 ± 3.56% of the initial current amplitude. Scale bars: 500 pA / 0.5 s

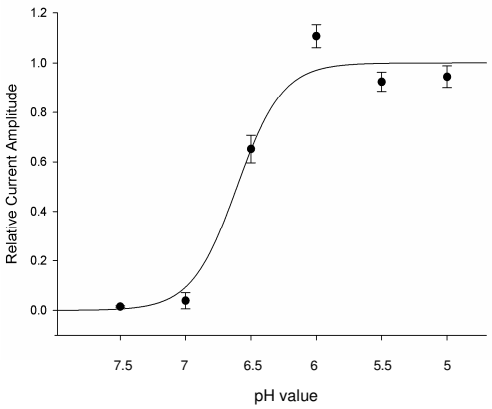


Summary of APETx2 experiments (n=7).

3.4 Automated Patch Clamping (Q-Patch):

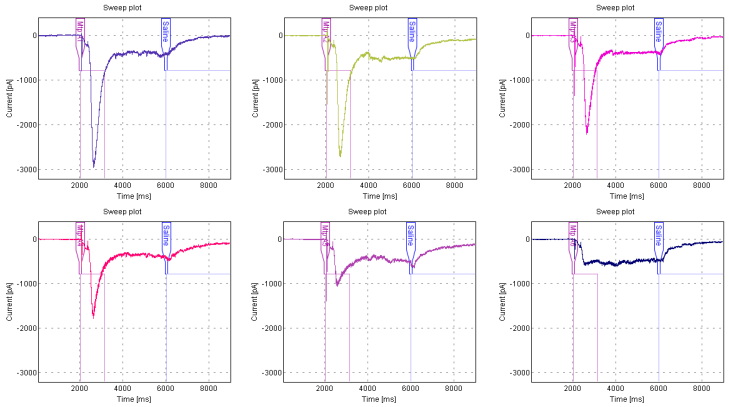
Cells were stimulated with solutions of decreasing pH (7.5 to 5, 0.5 increments). Between two applications the alkaline bath solution (pH8) was perfused for at least 30 s. The resulting pH_{50} was determined to 6.61 (n=5). This value is in good agreement with data from literature (Voilley and Lazdunski, 2007).

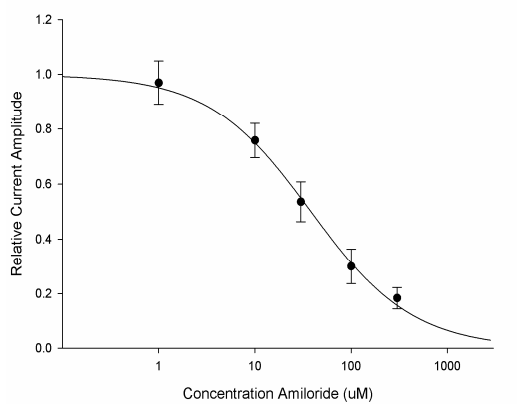




Bath solution pH 8, pH 7, pH 6.5, pH 6, pH 5.5, pH 5

The antagonist Amiloride hydrochloride was perfused together with bath solution of pH6.5 in increasing concentrations. Between two applications the alkaline bath solution (pH8) was perfused.





Bath solution pH 6.5, 1 µM, 10 µM, 30 µM, 100 µM, 300 µM Amiloride

4 CELL CULTURE CONDITIONS

4.1 General

CHO ASIC3 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% foetal bovine serum, 1% Penicillin/Streptomycin solution and 500 µg/mL Hygromycin. The CHO ASIC3 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 GlutaMAX I
- 10% FBS
- 1% Penicillin/Streptomycin

4.3 Antibiotics

- CHO ASIC3 clones were selected under 1000 µg/mL antibiotic pressure.
- To cultivate CHO ASIC3 cells, also a reduced antibiotic pressure (500 µg/mL) can be used.

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 vial containing 5 mL complete medium and centrifuge for 2 min.
- Remove the medium, add 5 mL complete medium to the vial and pipet carefully up and down to separate the cells.
- Passage the cell suspension into a new T-25 culture flask.
- Incubate cells at 37°C for 24 h, than add the antibiotics to the medium.
- Antibiotics: 500 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 cells for 2 min at 37°C.
- Detach cells, add complete medium to the cells and pipet up and down carefully.
- Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.

4.6 Freezing Medium

- Mix 0.9 mL fresh complete medium and 0.1 mL DMSO for every 1 mL freezing medium.

4.7 Freezing Cells

- 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 cells for 2 min at 37°C.
- Detach cells, add freezing medium (density of approximately 1.5 x 10^6 cells/mL).
- Aliquot 1 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.

5 ASIC3 SEQUENCE

5.1 Human ASIC3 Sequence

MKPTSGPEEARRPASDIRVFASNCSMHGLGHVFGPGSLSLRRGMWAAAVVLSVATFLYQVAERVRYYREF
HHQTALDERESHRLIFPAVTLCNINPLRRSRLTPNDLHWAGSALLGLDPAEHAAFLRALGRPPAPPGFMPSP
TFDMAQLYARAGHSLDDMLLDCRFRGQPCGPENFTTIFTRMGKCYTFNSGADGAELLTTTRGGMGNGLDI
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AIDAMLRKDSCACPNPCASTRYAKELSMVRIPSRAAARFLARKLNRSEAYIAENVLALDIFFEALNYETVEQKK
AYEMSELLGDIGGQMGLFIGASLLTILEILDYLCEVFRDKVLGYFWNRQHSQRHSSTNLLQEGLGSHRTQVP
HLSLGPRPPTPPCAVTKTLSASHRTCYLVTQL

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