

B'SYS GmbH

HEK P2X Cell Lines

Specification Sheet

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1 BACKGROUND

1.1 P2X Receptors

P2X receptors are a family of cation-permeable ligand gated ion channels that open in response to the binding of extracellular adenosine 5'-triphosphate (ATP). They belong to a larger family of receptors known as the purinergic receptors. The members of this class of ligand gated ion channels differ in their affinity to ATP and blockers and their biophysical properties.

1.2 B'SYS' P2X4 and P2X7 cell lines

The host of B'SYS' P2X cell lines are HEK293 cells. Cells were stably transfected and selected for high expression rates. They are suitable for manual and automated patch-clamping, as well for fluorescence based assays like FLEX station and FLIPR.

2 VALIDATION OF HEK P2X4 AND P2X7 CELLS

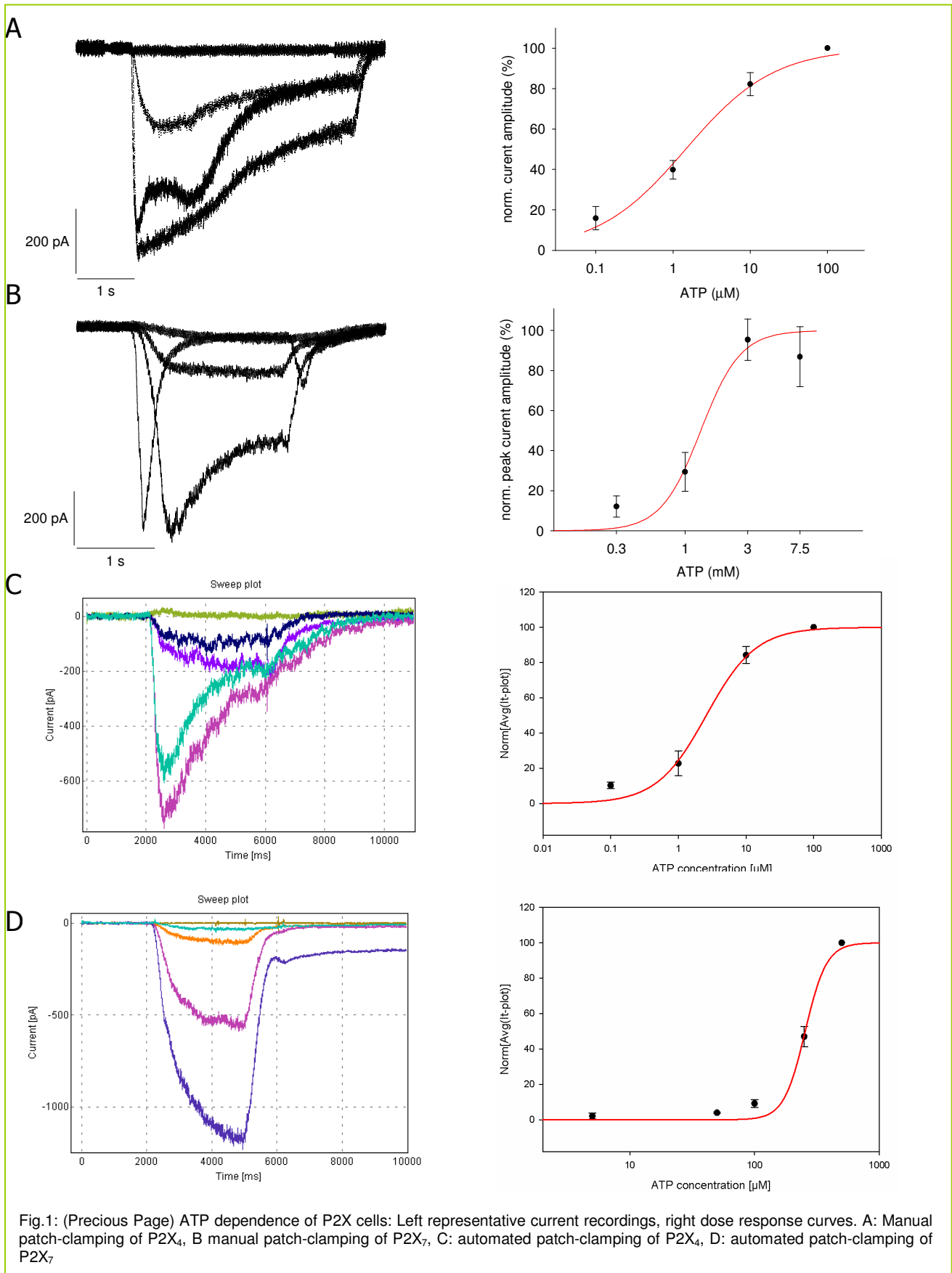
2.1 Electrophysiology

All cell lines were validated using manual patch-clamping and automated patch-clamping (Q-Patch). During validation, the dose dependence and the EC_{50} for ATP were determined. Further during a constant concentration of ATP, increasing concentrations of Suramine were applied and the IC_{50} calculated.

The extracellular solution was composed of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM Glucose, pH 7.4 (NaOH). The pipette solution contained: 130 mM KCl, 1 mM $MgCl_2$, 5 mM MgATP, 10 mM HEPES, 5 mM EGTA, pH 7.2 (KOH). Cells were clamped to -80 mV for the duration of the complete experiment. To accomplish fast solution exchanges, a 8 to 1 manifold was placed in close neighborhood of the tested cell.

The following EC_{50} for ATP were determined:

Cell Line	Method	EC_{50}
HEK P2X4	Manual patch-clamp	1.46 μ M
	Automated patch-clamp (Q-Patch)	2.59 μ M
HEK P2X7	Manual patch-clamp	1320 μ M
	Automated patch-clamp (Q-Patch)	282.76 μ M



The following IC_{50} for Suramine were determined (currents were evoked by ATP, concentrations given in Table):

Cell Line	Method	IC_{50} / ATP concentration
HEK P2X ₄	Manual patch-clamp	385.15 μ M / 2.00 μ M
HEK P2X ₇	Manual patch-clamp	100.95 μ M / 100 μ M

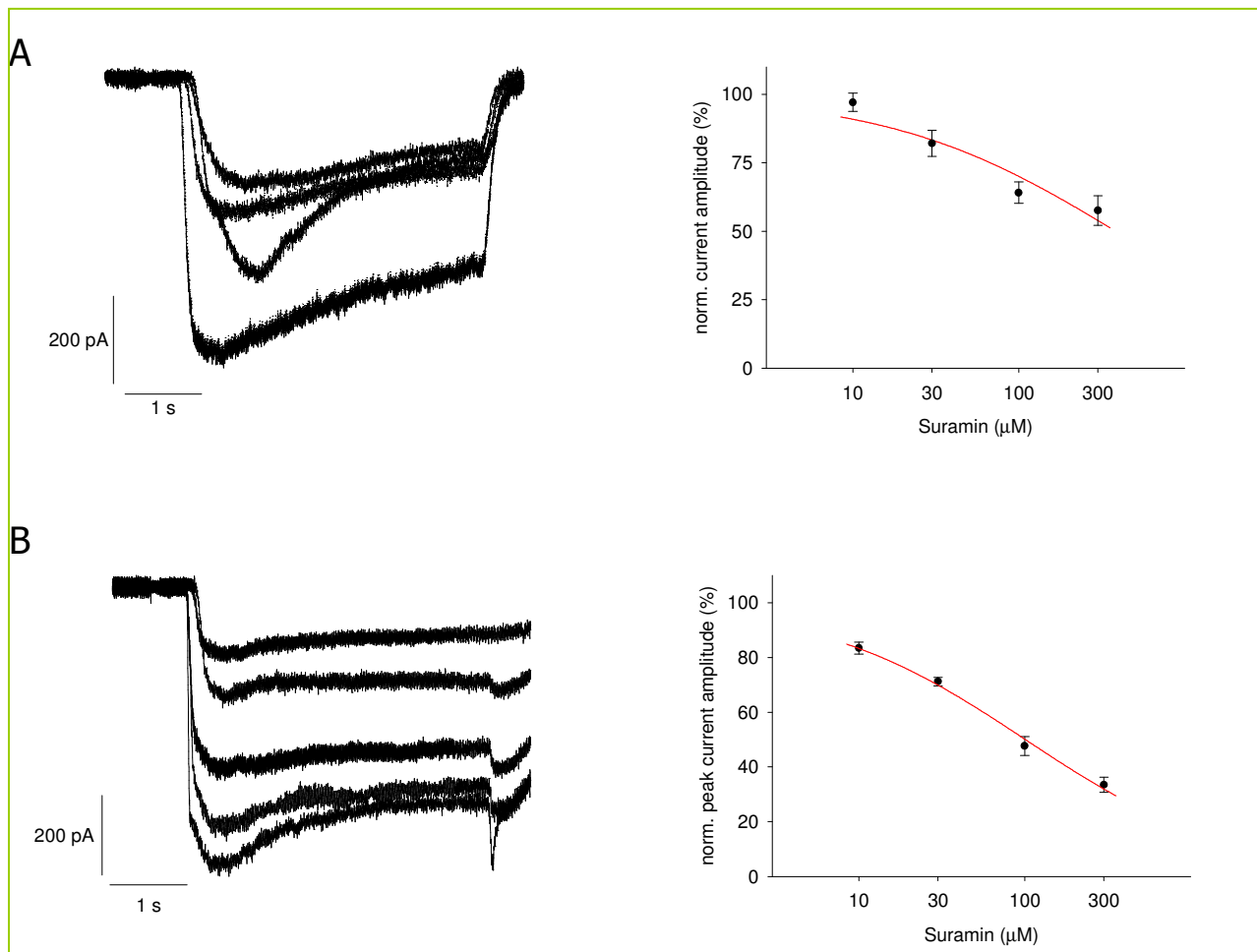


Fig.2: Suramin Block of P2X channels. Left: representative current recordings, left: Dose response curves. A: manual patch-clamping of P2X₄ cells, B: manual-patch-clamping of P2X₇ cells

2.2 Fluorescence based assays

The Calcium influx in HEK P2X7 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. Different ATP 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

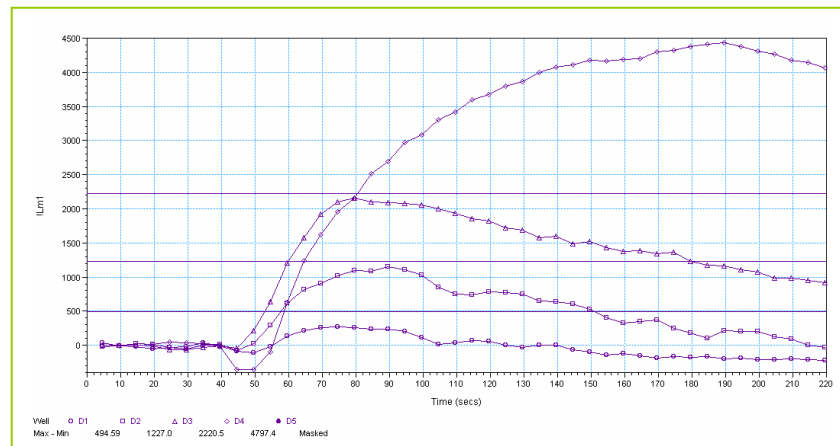


Fig. 3: Overlay of fluorescence signals recorded from wells treated with increasing concentrations of ATP.

3 CELL CULTURE CONDITIONS

3.1 General

HEK P2X 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 DMEM/F12 medium supplemented with 9% foetal bovine serum, 0.9% Penicillin/Streptomycin solution and 1.0 µg/mL Puromycin (P2X₄) or 100 µg/mL G-418 sulphate (P2X₇). The HEK P2X 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 splitted every 4-5 days at 50% - 80% confluency at 1:3 to 1:5 ratio.

3.2 Recommended Complete Medium

- DMEM/F12 with GlutaMAX I or L-Glutamine
- 9% FBS
- 0.9% Penicillin/Streptomycin

3.3 Antibiotics

- HEK P2X clones were selected under 3.0 µg/mL Puromycin (P2X₄) or 500 µg/ml G-418 sulphate (P2X₇) antibiotic pressure.
- To cultivate HEK P2X cells, a reduced antibiotic pressure should be used.

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

3.4 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: 1.0 µg/mL Puromycin (P2X₄) or 100 µg/mL G-418 sulphate (P2X₇)
- Incubate cells and check them daily until 50% - 80% confluency is reached.

3.5 Splitting Cells

- When cells are 50% - 80% confluent remove complete medium.
- Wash cells once with 1x Trypsin/EDTA.
- Remove Trypsin/EDTA quickly and incubate cells for 3-5 min at room temperature.
- 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.

3.6 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).

3.7 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 Trypsin/EDTA.
- Remove Trypsin/EDTA quickly and incubate cells for 3-5 min at room temperature.
- 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 3.0×10^6 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.

3.8 Stability of HEK P2X cells

HEK P2X cells stably express functionally active P2X channels over 20 passages. Under recommended cell culture conditions no variation in current density was observed over 20 cell splitting cycles.

4 CONTACT INFORMATION

4.1 Contact Address for Technical Support & Ordering Information

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