B'SYS GmbH

# CHO GlyR$\alpha_3$ Cell Line

Specification Sheet

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# 1 BACKGROUND

## 1.1 The GlyRα3 Receptors

Glycine is a major inhibitory neurotransmitter in the adult vertebrate central nervous system. Glycinergic synapses have a well-established role in the processing of motor and sensory information that controls movement, vision and audition. This action of glycine is mediated through its interaction with the glycine receptor, a chloride channel opens after glycine binding. The influx of anions prevents membrane depolarisation and neuronal firing induced by excitatory neurotransmitters. Strychnine acts as a competitive antagonist of glycine binding, thereby reducing the activity of inhibitory neurons. Compounds that modulate Glycine receptors activity include zinc, some alcohols and anaesthetics, picrotoxin, cocaine and some anticonvulsants. GlyRα3 is expressed in the cerebellum, olfactory bulb and hippocampus.

## 1.2 B'SYS' CHO GlyRα3 Cells

B'SYS has designed a new CHO GlyRα3 cell line with constitutive coexpression of human Glycine receptor alpha 3. The human GlyRα3 cDNA was cloned and transfected into CHO cells and then the functional properties of the GlyRα3 receptors 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 GlyRα3 channel:

- 1 x 0.5 mL aliquots of frozen cells at 2.2 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 GLYRA3 CELLS

## 3.1 Electrophysiology

GlyR$\alpha_3$ 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, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual GlyR$\alpha_3$ 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 currents were measured upon application of 150 µM Glycine at a holding potential of -80 mV (Fig. 1). The cell was stimulated after a minimum rest of 30 s.

## 3.2 Block by Strychnine

Cells were stimulated with 150 µM Glycine. Increasing concentrations of Strychnine were co-applied and the mean current amplitudes were plotted versus the Strychnine concentration. The dose response curve was generated and an $IC_{50}$ of 51.9 nM was determined (Hill coefficient: 1.34)

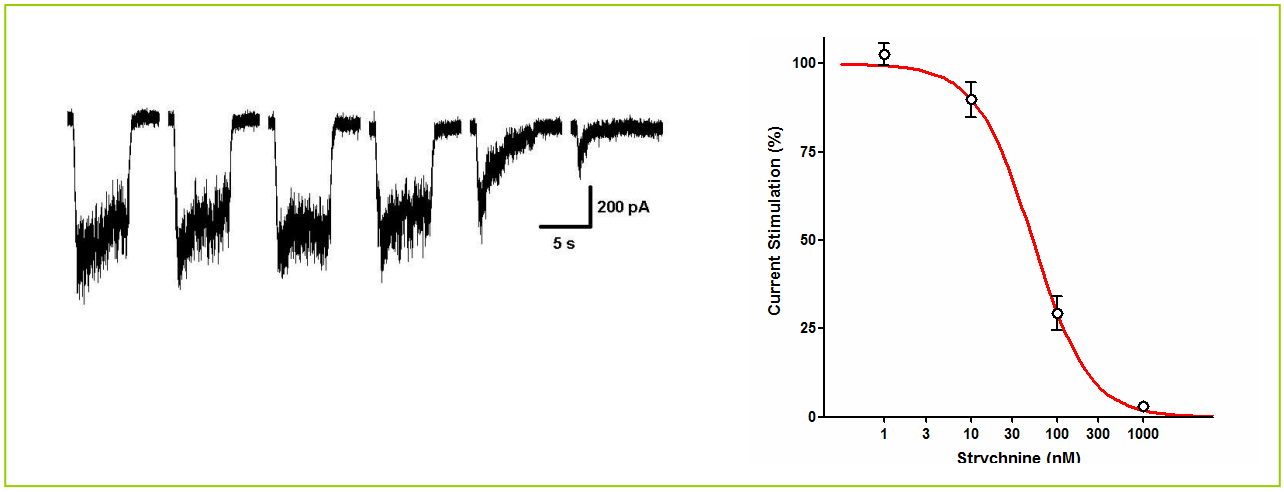


Fig.1: Strychnine block of GlyRα$_3$ currents. Cells were stimulated with 150 µM Glycine. Glycine was applied twice followed by increasing concentrations of Strychnine: 1.0, 10, 100 and 1000 nM dissolved in bath solution (stock solution of 1.0 mM).

# 4 CELL CULTURE CONDITIONS

## 4.1 General

CHO GlyR$\alpha_3$ 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 100 µg/mL G-418 suplhate. The CHO GlyR$\alpha_3$ 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 2-3 days at 70% - 80% confluency at 1:3 to 1:5 ratio.

## 4.2 Recommended Complete Medium

- F12 (HAM) with GlutaMAX I
- 9% FBS
- 0.9% Penicillin/Streptomycin

## 4.3 Antibiotics

- CHO GlyR$\alpha_3$ clones were selected under 1000 µg/mL G-418 suplhate antibiotic pressure.
- To cultivate CHO GlyR$\alpha_3$ cells, also a reduced antibiotic pressure (100 µg/mL) can be used.
- To separate CHO GlyR$\alpha_3$ cells from untransfected cells, use 1000 µg/mL G-418 suplhate.

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 10 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 10 mL complete medium & antibiotics.
- Antibiotics: 100 µg/mL G-418 suplhate.
- 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 (without $Mg^{2+}$ and $Ca^{2+)}$ 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 (0.1 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.
- Wash cells once with 1x PBS (without $Mg^{2+}$ and $Ca^{2+}$) 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.

### 4.8 Stability of CHO GlyRα3 cells

CHO GlyRα$_3$ cells stably express functionally active GlyRα$_3$ potassium channels over 25 passages. Under recommended cell culture conditions no variation in current density was observed over 25 cell splitting cycles.

## 5 CONTACT INFORMATION

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