B'SYS GmbH

# CHO GlyR$\alpha_1$ Cell Line

Specification Sheet

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# 1 BACKGROUND

## 1.1 The GlyR$\alpha_1$ 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. GlyR$\alpha_1$ are highly expressed in the pons, medulla, midbrain and hypothalamus and mutations in the receptor have been shown to cause the dominant neurologic disorder hyperekplexia.

## 1.2 B'SYS' CHO GlyR$\alpha_1$ Cells

B'SYS has designed a new CHO GlyR$\alpha_1$ cell line with constitutive coexpression of human Glycine receptor alpha 1. The human GlyR$\alpha_1$ cDNA was cloned and transfected into CHO cells and then the functional properties of the GlyR$\alpha_1$ 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 GlyR$\alpha_1$ 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 GLYRA1 CELLS

## 3.1 Electrophysiology

GlyRα2 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 GlyRα1 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. Currents were measured by applying glycine for 4 seconds at a holding potential of -80 mV (Fig. 1). Between each agonist application, the cell was perfused with bath solution for 60 s.

## 3.2 Concentration-response curve to Glycine

Cells were stimulated with increasing, cumulative concentrations of 3, 10, 30, 100 and 300 µM Glycine. The mean current amplitudes were plotted against the Glycine concentration. The dose response curve was generated and an $EC_{50}$ of 45.19 µM was determined (Hill coefficient: 5.09)

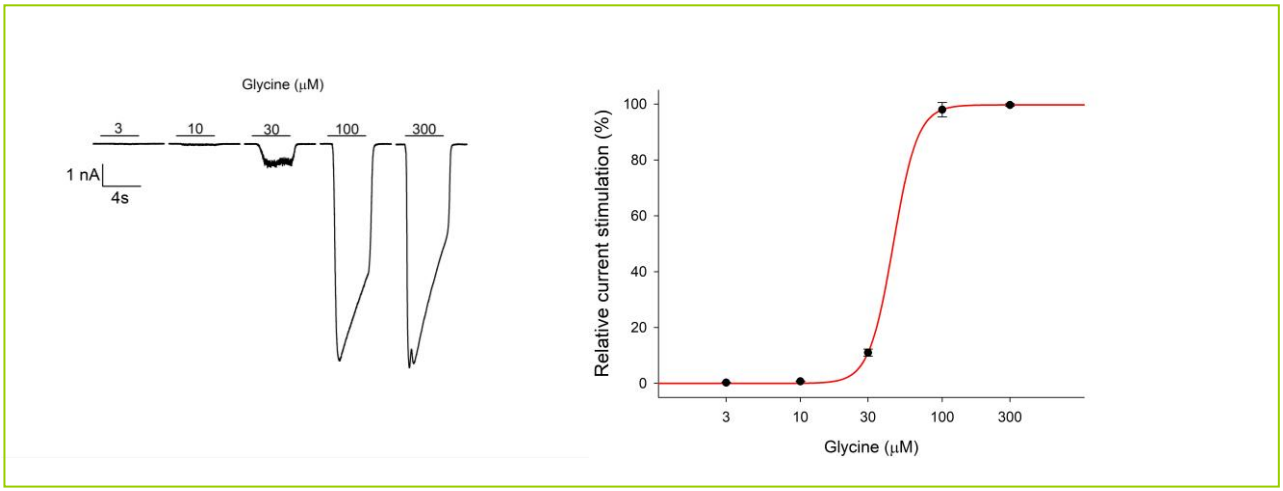


Fig.1: Concentration-response curve to Glycine on GlyRα1-expressing cells. Cells were stimulated with cumulative, increasing concentrations of Glycine.

### 3.3 Block by Strychnine

Cells were stimulated with 45 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 66.82 nM was determined (Hill coefficient: 1.38)

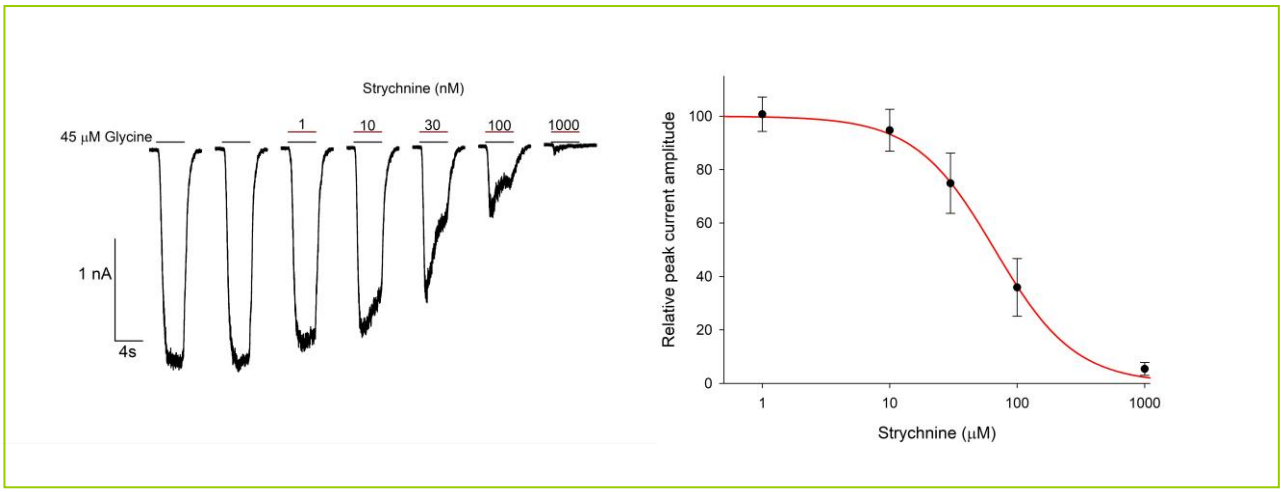


Fig.2: Strychnine block of GlyRα1 currents. Cells were stimulated with 45 µM Glycine. Glycine was applied twice followed by increasing concentrations of Strychnine: 1.0, 10, 30, 100 and 1000 nM dissolved in bath solution (stock solution of 1.0 mM).

# 4 CELL CULTURE CONDITIONS

## 4.1 General

CHO GlyRα1 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% Penicillin/Streptomycin solution and 1 µg/mL Puromycin and 15 µg/mL Blasticidin. The CHO GlyRα1 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.

**For the induction of GlyRα1 channel expression 2.5 µg/mL Tetracycline has to added 24-48 h before experimentation.**

- 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.

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 |
| Puromycin (10 mg/mL) | Gibco | A1113803 |
| Blasticidin S HCl | Gibco | R21001 |
| Detachin | Genlantis | T100T100 |
| Trypsin EDTA (10X) | Sigma-Aldrich | T4174 |
| DMSO | Sigma-Aldrich | D2438 |
| Distilled water ($dH_2O$) | Gibco | 15230204 |
| Tetracycline hydrochloride | Sigma Aldrich | T7660 |

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.

For the preparation of blasticidin, the powder is diluted in $dH_2O$ to achieve a stock concentration of 15 mg/mL, filter sterilized and stored frozen.

For the preparation of tetracycline, the powder is diluted in $dH_2O$ to achieve a stock concentration of 1 mg/mL, filter sterilized, aliquoted and stored in the freezer. (Do not store for more than 6 months!)

## 4.2 Recommended Complete Medium

- 500 mL F12 (HAM) with L-Glutamine or GlutaMax I
- 10% FBS
- 1% Penicillin/Streptomycin

### 4.3 Antibiotics

- CHO GlyRα1 clones were selected under 3 µg/mL Puromycin and 15 µg/mL Blasticidin antibiotic pressure.
- To cultivate CHO GlyRα1 cells, also a reduced antibiotic pressure 1 µg/mL Puromycin and 15 µg/mL Blasticidin can be used.

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 to allow the cells to attach to the bottom of the flask
- Once cells attach to the bottom of the flask, look healthy and proliferate, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see **Error! Reference source not found.**)
- 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.
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### 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.
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### 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.

### 4.8 Induction of GlyRα1 Receptor Expression

For the induction of GlyRα1 receptor expression, 2.5 µg/mL Tetracycline has to be added 24 to 48 h before experimentation.

### 4.9 Stability of CHO GlyRα1 cells

CHO GlyRα1 cells stably express functionally active GlyRα1 channels over 25 passages. Under recommended cell culture conditions, no variation in current density was observed over 25 cell splitting cycles.

## 5 GlyRα1 SEQUENCE

CHO GlyRα1 cells have been stably transfected with codon optimized cDNA encoding for the human GlyRα1 receptor (NP_001139512.1) by non-viral lipofection.
The expression of the GlyRα1 receptor is under the control of a CMV promoter. For amplification in e-coli, an Ampicillin resistance gene is located on the complementary strand under the control of a bla promoter.

```
MYSFNTLRLYLWETIVFFSLAASKEAEAARSAPKPMSPSDFLDKLMGRTSGYDARIRPNFKGPPVNVSCNIFINSFGSIA
ETTMDYRVNIFLRQQWNDPRLAYNEYPDDSLDLDPSMLDSIWKPDLFFANEKGAHFHEITTDNKLLRISRNGNVLYSIRI
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PPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ*
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## 6 CONTACT INFORMATION

### 6.1 Contact Address for Technical Support & Ordering Information


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