B'SYS

B’SYS GmbH

CHO $\alpha_1\beta_2\gamma_2$ $GABA_A$ Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The Pharmacological Distinction of $GABA_A$ Receptor Subtypes

Using genetically modified (knock-in) mice it has been demonstrated that $GABA_A$ receptors containing an α1 subunit mediate the sedative/muscle relaxant effects of benzodiazepines, whereas β_2 and/or β_3 subunit containing receptors mediate the anxiolytic and anticonvulsant effects. $GABA_A$ α_5 receptors have a relatively restricted distribution being primarily expressed in the hippocampus, a region of the brain associated with learning and memory, and although α_5 receptors account for less than 5% of the total $GABA_A$ receptor population in the brain, in the hippocampus they represent 20% of all $GABA_A$ receptors, thereby implicating this $GABA_A$ receptor subtype in learning and memory processes. Thus, the pharmacological distinction of $GABA_A$ receptor isoforms serves now as a promising basis for the development of new agents effective at only restricted brain regions and thus exhibiting unique and specific physiological effects.

1.2 B'SYS' CHO $\alpha_1\beta_2\gamma_2$ Cells

B'SYS has designed a CHO $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor cell line with constitutive expression of human $\alpha_{?}$ subunit together with the β_2 and γ_2 subunits. The human $GABA_A$ receptor cDNAs were cloned and transfected into CHO cells and then the functional properties of the $GABA_A$ receptors validated by means of manual and automated (Q-Patch) patch-clamp technique. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant $\alpha_1\beta_2\gamma_2$ human $GABA_A$ receptors:

- 0.75 mL aliquots of frozen cells at approximately 3.0 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 $\alpha_1\beta_2\gamma_2$ $GABA_A$ CELLS

3.1 Electrophysiology

$GABA_A$ 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 $\alpha_1\beta_2\gamma_2$ $GABA_A$ 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 maintained at room temperature. The holding potential was -80 mV. As soon as a stable seal could be established inward chloride currents were measured upon application of agonist concentrations (GABA) to patch-clamped cell.

3.2 GABA as Agonist

The concentration dependence for GABA was tested using automated patch-clamping (Q-Patch). Concentrations between 0.01 µM and 100 µM were applied. Increasing concentrations were tested every 30 s. GABA was washed off after each GABA application. The EC_{50} was determined to be 5.92 µM (Hill coefficient 2.4).

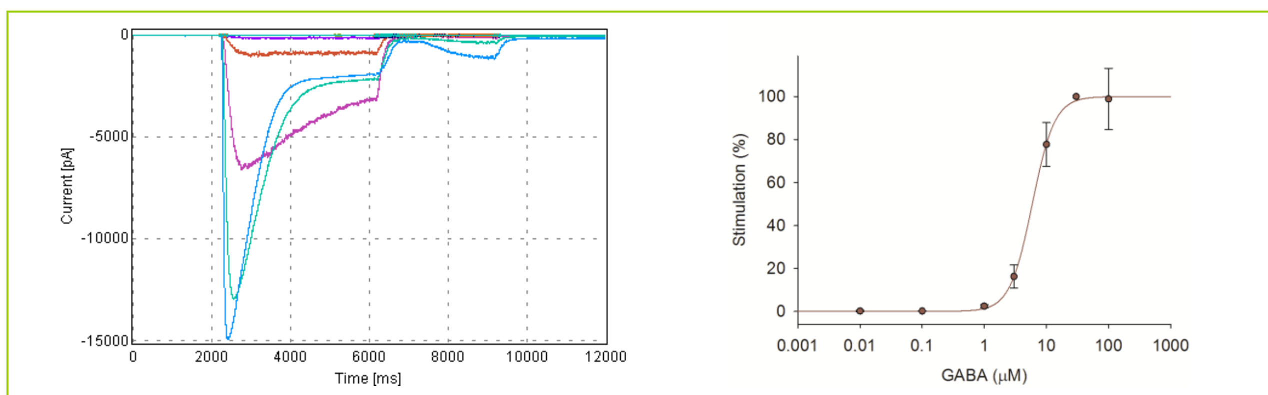

Fig.1: Activation of $GABA_A$ receptor currents upon brief applications of increasing concentrations of GABA to patch-clamped cell. The cell membrane was hold at -80 mV. No inward chloride currents were recorded in untransfected cells (data not shown).

3.3 Positive allosteric agonists (PAM)

As positive allosteric agonists Diazepam and Propofol were tested in concentrations between 0.01 µM and 30 µM in the presence of 2.0 µM GABA:

Diazepam: EC_{50}: 0.11 µM, Hill: 1.29, a_{max}: 152%

Propofol: EC_{50}: 3.59 µM, Hill: 1.31, a_{max}: 763%

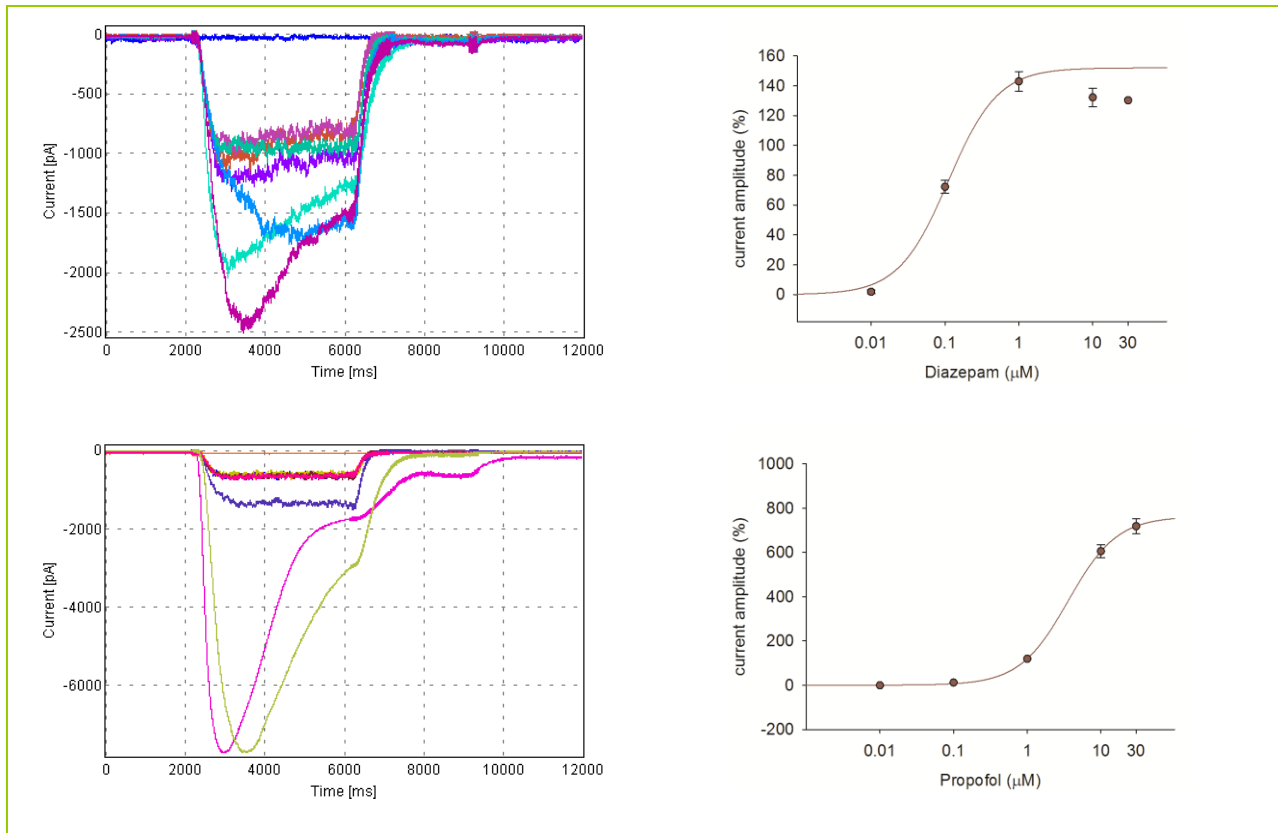

Fig.2: Effects of positive allosteric modulators (in the presence of 2.0 µM GABA): Top Diazepam, Bottom Propofol

3.4 Antagonist

As antagonist Bicuculline was tested at concentration between 0.01 µM and 10 µM in the presence of 5.0 µM GABA. The IC_{50} was determined to be: 0.48 µM (Hill coefficient: 0.92).

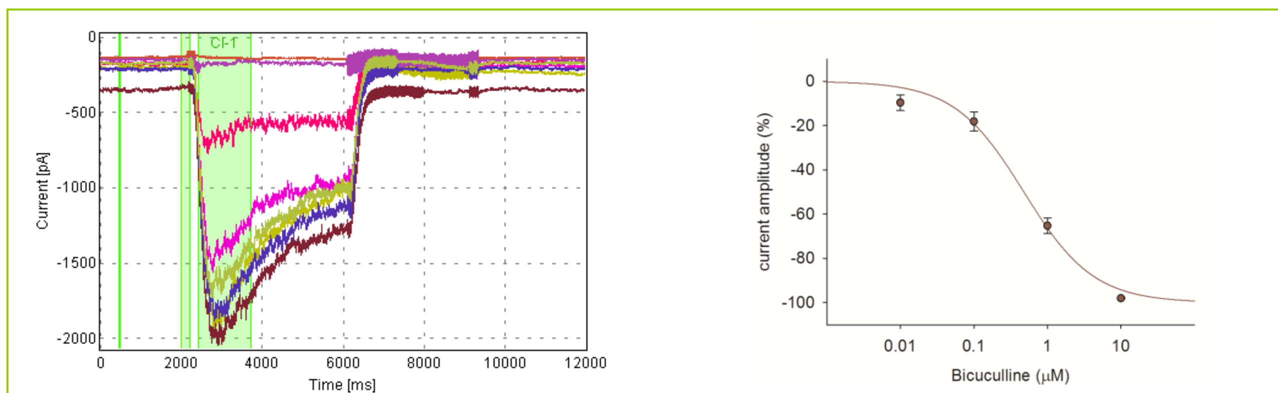

Fig.3: Effects of the antagonist Bicuculline (in the presence of 5.0 µM GABA)

3.5 Patch-clamp Success Rates

The patch-clamp properties of the CHO $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor cell line were elucidated at typical working passage numbers (passage 2-18). A total of 63 cells were analyzed. Success for establishment of on-cell configuration was defined as follows: > 1 GΩ. The whole-cell configuration was not accepted if the membrane resistance was below 500 MΩ. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **95%** (n=80)
- Whole-cell successful: **91%** (n=80)
- Recording (15 min) successful: 7**8%** (n=80)

4 CELL CULTURE CONDITIONS

4.1 General

CHO $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor 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, 1.0% Penicillin/Streptomycin solution and Hygromycin 250 µg/mL, Puromycin 5 µg/mL, Zeocin 100 µg/mL. The CHO $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor cells are passaged at a confluence of about 50 to 80%.

- 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% to 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
Zeocin (100 mg/mL)	Gibco	R25001
Detachin	Genlantis	T100T100
Trypsin EDTA (10x)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438

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.

4.2 Recommended Complete Medium

- 500 mL F12 (HAM) with L-Glutamine
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- CHO $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor clones were selected under Hygromycin 500 µg/mL, Puromycin 5 µg/mL, Zeocin 100 µg/mL antibiotic pressure.
- To cultivate $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor cells, a reduced antibiotic pressure (Hygromycin 250 µg/mL, Puromycin 5 µg/mL, Zeocin 100 µg/mL) should be used.

Remark: The permanent application of 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 at least 4-6 hours to allow the cells to attach to the bottom of the flask
- Once cells attach to the bottom of the flask and look healthy, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see 4.3)
- 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% - 80% confluency is reached.

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.

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.

5 SEQUENCE

Some subunits were codon optimized.

5.1 GABA α_1, NP_000797.2

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MRKSPGLSDCLWAWILLLSTLTGRSYGQPSLQDELKDNTTVFTRILDRLLDGYDNRLRPGLGERVTEVKTDIFVTSFGPV
SDHDMEYTIDVFFRQSWKDERLKFKGPMTVLRLNNLMASKIWTPDTFFHNGKKSVAHNMTMPNKLLRITEDGTLLYTMRL
TVRAECPMHLEDFPMDAHACPLKFGSYAYTRAEVVYEWTREPARSVVVAEDGSRLNQYDLLGQTVDSGIVQSSTGEYVVM
TTHFHLKRKIGYFVIQTYLPCIMTVILSQVSFWLNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVC
YAFVFSALIEFATVNYFTKRGYAWDGKSVVPEKPKKVKDPLIKKNNTYAPTATSYTPNLARGDPGLATIAKSATIEPKEV
KPETKPPEPKKTFNSVSKIDRLSRIAFPLLFGIFNLVYWATYLNREPQLKAPTPHQ*
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5.2 GABA β_2, NP_000804.1

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MWRVRKRGYFGIWSFPLIIAAVCAQSVNDPSNMSLVKETVDRLLKGYDIRLRPDFGGPPVAVGMNIDIASIDMVSEVNMD
YTLTMYFQQAWRDKRLSYNVIPLNLTLDNRVADQLWVPDTYFLNDKKSFVHGVTVKNRMIRLHPDGTVLYGLRITTTAAC
MMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGDDNAVTGVTKIELPQFSIVDYKLITKKVVFSTGSYPRLSLSFKLKR
NIGYFILQTYMPSILITILSWVSFWINYDASAARVALGITTVLTMTTINTHLRETLPKIPYVKAIDMYLMGCFVFVFMAL
LEYALVNYIFFGRGPQRQKKAAEKAASANNEKMRLDVNKMDPHENILLSTLEIKNEMATSEAVMGLGDPRSTMLAYDASS
IQYRKAGLPRHSFGRNALERHVAQKKSRLRRRASQLKITIPDLTDVNAIDRWSRIFFPVVFSFFNIVYWLYYVN*
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5.3 GABA γ_2, NP_000807.2

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MSSPNIWSTGSSVYSTPVFSQKMTVWILLLLSLYPGFTSQKSDDDYEDYASNKTWVLTPKVPEGDVTVILNNLLEGYDNK
LRPDIGVKPTLIHTDMYVNSIGPVNAINMEYTIDIFFAQTWYDRRLKFNSTIKVLRLNSNMVGKIWIPDTFFRNSKKADA
HWITTPNRMLRIWNDGRVLYTLRLTIDAECQLQLHNFPMDEHSCPLEFSSYGYPREEIVYQWKRSSVEVGDTRSWRLYQF
SFVGLRNTTEVVKTTSGDYVVMSVYFDLSRRMGYFTIQTYIPCTLIVVLSWVSFWINKDAVPARTSLGITTVLTMTTLST
IARKSLPKVSYVTAMDLFVSVCFIFVFSALVEYGTLHYFVSNRKPSKDKDKKKKNPAPTIDIRPRSATIQMNNATHLQER
DEEYGYECLDGKDCASFFCCFEDCRTGAWRHGRIHIRIAKMDSYARIFFPTAFCLFNLVYWVSYLYL*
```

6 CONTACT INFORMATION

6.1 Contact Address for Technical Support & Ordering Information

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