PRODUCT DATASHEET

PrecisION™ hGABA\textsubscript{A} α1/β3/γ2-HEK Recombinant Cell Line

Catalog Number: CYL3053

PRODUCT DESCRIPTION
Recombinant HEK293 cell line expressing the human GABA\textsubscript{A} α1, β3 and γ2 subunits.

ASSOCIATED PRODUCTS
The PrecisION™ hGABA\textsubscript{A} α1β3γ2-HEK Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:
- CYL3053SS: PrecisION™ hGABA\textsubscript{A} α1β3γ2-HEK
- CYL3053TS: PrecisION™ hGABA\textsubscript{A} α1β3γ2-HEK
- CYL3053MS: PrecisION™ hGABA\textsubscript{A} α1β3γ2-HEK

CONTENTS
2 x 1 mL aliquots containing 2.2 x 10\textsuperscript{6} cells/mL in 10% DMSO at passage 15.

STORAGE
Vials are to be stored in liquid N\textsubscript{2}.

WARNINGS
For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

MYCOPLASMA TESTING
The cell line has been screened using the ELISA based Mycoplasma Detection kit (Roche) and by a PCR VenorGem kit (Minerva Biolabs) to confirm the absence of Mycoplasma species.
FUNCTIONAL VALIDATION
HEK293 cells expressing human GABA_\textsubscript{A} α1, β3 and γ2 subunits were characterized in terms of their pharmacological and biophysical properties using manual patch clamp techniques (perforated patch-clamp and conventional whole-cell recording) and automated planar array electrophysiology - IonFlux™ HT and IonWorks™ Quattro.

Using manual patch clamp techniques, 1 mM GABA produced a mean peak current amplitude of 2.21 ± 0.28 nA (mean ± SEM, n=10). The mean current density was 176.5 ± 26.5 pA/pF (mean ± SEM, n=10).

The GABA concentration response relationships yielded EC\textsubscript{50} values of 3.32 µM using the IonFlux™ platform, and 1.96 µM (mean, n=16) using the IonWorks™ platform. Peak currents were measured on the IonFluxTM platform, while IonWorksTM measured steady state activation at increasing agonist concentrations. Two additional GABA receptor agonists, receptor agonists, muscimol and isoguavacine, were tested on the IonFlux instrument with EC50 values of 1.71 ± 0.3 µM and 11.2 ± 1.0 µM respectively.

Bicuculline, a known GABA\textsubscript{A} antagonist, abolished (>98% block at 11 µM) responses to 10 µM GABA. This antagonism was demonstrated to be competitive, with a pA\textsubscript{2} value of 6.1. The GABA blocker picrotoxin produced inhibition of the 30 µM GABA response with a mean IC\textsubscript{50} value of 3.2 µM.

The benzodiazepines pentobarbital, tracazolate and zolpidem all positively modulated the 1 µM GABA response, by at least 300%, confirming the presence of the γ2 subunit. Using manual patch clamp techniques, measurement of the zolpidem potentiation of GABA response showed this subunit expression to be stable over time.

Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 32 passages. At passage 32, 83% of cells sealed with a resistance >50 MOhm. Of these, 94% expressed hGABA\textsubscript{A} α1/β3/γ2 currents >500 pA with a mean current amplitude of 2.76 nA ± 0.21 nA (n=183). In addition, the channel stability was tested over 36 passages on the IonFlux™. Successful functional channel expression was defined as a current greater than 2 nA elicited in response to challenge with 5µM GABA. The success rate was greater than 95% over all passages tested.

RECOMMENDED CULTURE CONDITIONS
Recommended culture conditions and standard operating procedure are provided with the product.
FUNCTIONAL VALIDATION DATA

Electrophysiological Properties of the hGABA_A Current.

Conventional Whole-Cell Patch Clamp Electrophysiology.

GABA_A receptors are ligand-gated, multi-subunit Cl⁻ ion channels, and are the major inhibitory neurotransmitter receptors in the CNS. The presence of different subunits confers different pharmacological, modulatory and functional properties for each receptor subtype. The largest population of GABA_A receptors in mammalian brain have been shown to contain the α1 subunit. Classical benzodiazepines act on GABA_A receptors that contain a γ2-, a β and an α1, α2, α3 or α5-subunit. Undesirable side effects (such as sedation, amnesia and ataxia) appear to be mediated by α1 subunit-containing receptors.

Membrane currents were recorded using either perforated patch-clamp or conventional whole-cell recording. A number of experiments were also performed using high-throughput planar array electrophysiology (IonWorks™ Quattro).

For conventional patch clamp the internal solution contained (mM) 150 CsCl, 2 EGTA, and 10 HEPES, pH 7.3 (with CsOH). For perforated patch-clamp recordings, pipettes were backfilled with internal solution containing 240 µg/ml amphotericin B. The external solution was (mM) 140 NaCl, 2 KCl, 1 MgCl₂, 2 CaCl₂, 10 glucose, and 12 HEPES, pH 7.35 (with NaOH). Agonists were applied using fast perfusion systems. The holding potential was -60 mV throughout the experiment. Full experimental details for assays using the IonFlux instrument can be found at www.fluxionbio.com/library-c2-IonFlux_System.aspx.

Peak Current Analysis:
The effect of 1 mM GABA (Gamma-Aminobutyric Acid) on HEK293 cells stably expressing hGABA_A α1/β3/γ2 was tested using conventional patch clamp techniques. All cells tested responded to GABA application. The mean peak current amplitude was 2.21 ± 0.28 nA (mean ± SEM, n=10). In order to allow for different cell sizes, the data can also be expressed as a mean current density; 176.5 ± 26.5 pA/pF (mean ± SEM). An example of recorded current is shown in Figure 1. The distribution of peak current amplitudes and densities are shown in Figure 2.

Figure 1. Typical current trace on application of 1 mM GABA.
Figure 2. Peak current amplitude and peak current density distributions.
Data obtained from patch clamp recordings from hGABA_A α1/β3/γ2-HEK293 cells (n=10, bin sizes 200 pA and 30 pA/pF respectively).

**Pharmacological Properties of the hGABA_A Current.**

**IonFlux™ Automated Patch Clamp Recordings**
IonFlux™ is a next generation automated electrophysiology instrument based on a microfluidic platform for compound delivery and cell handling. Because operation is independent of fluidic handlers, continuous recording is achieved during compound additional and removal, and additions are simultaneous across the entire well plate, resulting in dramatically shorter experimental timescales as compared to systems that are based on robotic fluid handling during the experiment. In order to characterize the agonist response of the GABAA α1 channel, three different agonists were applied in increasing concentration, and Hill fits determined the EC50 value for each dose response curve (Figure 2). All EC50 measurement are in good agreement with literature values.

**Figure 3. IonFlux™ concentration-response curves for GABA_A α1 agonists.**

A: Example of typical GABA_A α1 currents activated by increasing concentrations of isoguvacine, a GABA receptor agonist, applied for 3 s at increasing doses from 1μM to 100μM.

B: Comparison of dose-response curves for three agonists: GABA, isoguvacine, and muscimol. Hill fits yield EC50 values of 3.3, 11.2, and 1.7 μM respectively, in good agreement with literature.
Figure 4. Positive allosteric modulator response

**A:** Response of a cell ensemble exposed to 1 μM GABA (EC20) with pre-incubated and co-applied with increasing diazepam concentrations.

**B:** Dose response analysis yielded EC50 of diazepam 425nM (n=4, hill slope = 1.28, R2= 0.977). The reported literature EC50 value is ~160nM. EC50 of zolpidem was 84.6nM (n=3, hill slope = 1.25 R2=0.967). The reported literature EC50 values range from 70nM to 150nM. EC50 of triazolam was 12.1nM (n=4, hill slope =1.41, R2=0.970). The reported literature EC50 values range from 22nM to 45nM.
IonWorks™ Quattro.

For the planar array experiments a low Cl⁻ (10 mM) based internal and high Cl⁻ (140 mM) based external recording solution was employed. GABA responses were measured 30–40 s post application, i.e. not at the peak.

For planar array, the GABA concentration response relationship yielded a mean EC$_{50}$ value of 1.96 µM (n=16) and a slope (k) of 2.0 (Figure 3A) – these values are almost certainly overestimated compared to the true values that would be obtained if peak currents were resolved. Bicuculline, a known GABA antagonist, abolished (>98% block at 11 µM) responses to 10 µM GABA. It was also further demonstrated that a single dose (100 µM) of bicuculline completely inhibited the currents across a full GABA dose response curve (Figure 4). Further analysis of this interaction demonstrated that the antagonism was competitive, with a pA$_2$ value of 6.1 and slope of 1.0. A second GABA$_A$ blocker, picrotoxin, produced inhibition of the 30 µM GABA response with a mean IC$_{50}$ value of 3.2 µM (n=16) and slope of 1.2. (Figure 3B).

Figure 5. Agonist and antagonist dose response curves (n=16, mean ± SEM).

A. GABA activation.

B. Picrotoxin inhibition of the 30 µM GABA response.
In addition it was shown that the benzodiazepine zolpidem potentiated the 1 µM GABA signal with an approximate EC$_{50}$ of 2 µM (Figure 5). This positive modulation was blocked by 10 µM flumazenil. Positive modulation of the 1 µM GABA response was also observed with pentobarbital, tracazolate and zolpidem with pEC$_{50}$ and maximal augmentation values (mean ± SEM) of 4.5 ± 0.2 (n=10), 5.4 ± 0.1 (n=2) and 6.8 ± 0.2 (n=10) and 378 ± 48%, 811 ± 81%, 571 ± 99%, respectively.

Figure 7. Potentiation of GABA response by zolpidem and block by flumazenil.
Conventional Whole-Cell Patch Clamp Electrophysiology.

In order to verify the co-expression of the γ2 subunit, the benzodiazepine midazolam was tested on the currents evoked by GABA (1 µM, EC\textsubscript{20}) using conventional patch clamp. 1 µM midazolam augmented this GABA response by 462 ± 88% (mean ± SEM - Figure 6). The 1 µM GABA response was also augmented by diazepam (Figure 6).

**Figure 8.** Potentiation of hGABA\textsubscript{A} mediated currents by the benzodiazepines midazolam and diazepam.

![Graph showing potentiation of hGABA\textsubscript{A} mediated currents by midazolam and diazepam.](image)

Zolpidem potentiation (approximately 2-3 fold) of the 1 µM GABA response was observed up to passage 25 (Figure 7), demonstrating that the channel is not changing its stoichiometry over passage and that the γ2 subunit is stably expressed along with the α1 and β3 subunits in this cell line.

**Figure 9.** Stability of γ2 subunit expression over passage.

![Bar graph showing Zolpidem potentiation over passages](image)
Stability of hGABA$_{\alpha1/\beta3/\gamma2}$-HEK293 Cell Line.

**IonFlux™ Electrophysiology**

The repeatability of the GABA response at sub-maximal concentrations is important to the development of screening protocols for modulator molecules. To that end, a large number of experiments were used to monitor the success rate (current peak above a 2nM threshold) in responses to a 5uM GABA application, as well as the average response amplitude at the same GABA concentration. The averaged results (categorized by passage number) are presented in Figure 3 from 60 IonFlux experiments and a corresponding 2606 recordings from 20-cell ensembles. The success rate (good signal to noise at sub-maximal concentrations) is very high, largely above 98%, with an average current amplitude that remains in the 5nM – 15nM range for verified performance passage numbers 18 to 36.

**Figure 10. IonFlux™ - success rates and current stability at the EC50 concentration**

Measurement of the success rate for GABA experiments, where success is defined as being able to elicit responses above 2nA to an EC50 GABA concentration (5uM) for the duration of the experiment – red line, right axis. Success rates were above 95% for all experimental runs spanning passages 18 to 36. Corresponding average current per channel, and standard error values for 57 IonFlux experiments (n=2606 total ensemble recordings, comprised of a combination of IonFlux 16 and IonFlux HT experiments) – blue bar graph, left axis.
IonWorks™ HT Electrophysiology.

The hGABA_α1/β3/γ2-HEK293 cell line has stable expression for >32 passages.

Functional channel expression, defined as cells expressing hGABA current of ≥ 500 pA, was monitored using IonWorks™ HT. This data and the mean current amplitude is shown in Figure 11. Sealing data is shown in Figure 12.

**Figure 11. IonWorks™ - stability of expression over passage.**
The upper panel shows the percentage of cells expressing a mean peak current >500 pA at 0 mV at cell passages 1, 11, 17, 21, 26 and 32. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles - out of 32 cells for passages 1 & 11 and out of 64 cells for all other passages).

**Figure 12. Sealing rates over passage.**
The percentage of cells sealing (defined by a seal resistance of >50 MOhm).
Vectors:

Polylinker: CMV-BamHI-NotI-α1-EcoRI-IRES-neo
Polylinker: CMV-BamHI-BstXI-β3-NotI-EcoRI-IRES-hyg
Polylinker: CMV-NotI-γ2-NotI-EcoRI-BamHI-BstXI-IRES-puro

hGABA_A Sequences:
α1 (Accession Number NM_000806): The sequence of the cDNA used to make this cell line contains one silent mutation with respect to the Accession Number (NM_000806) GGT-GGC (Val) - at position 156.

β3 (Accession Number NM_000814).

γ2 (Accession Number NM_000816).
REFERENCES


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