PRODUCT DATASHEET

PrecisION™ hGABA_α4/β3/γ2-HEK Recombinant Cell Line

Catalog Number: CYL3085

PRODUCT DESCRIPTION
Recombinant HEK293 cell line expressing the human GABA_α4, β3 and γ2 subunits.

ASSOCIATED PRODUCTS
The PrecisION™ hGABA_α4/β3/γ2-HEK Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:
- CYL3085SS  PrecisION™ hGABA_α4/β3/γ2-HEK Single Site License
- CYL3085TS  PrecisION™ hGABA_α4/β3/γ2-HEK Two Site License
- CYL3085MS  PrecisION™ hGABA_α4/β3/γ2-HEK Multiple Site License

CONTENTS
2 x 1 mL aliquots containing 3.0 × 10^6 cells/mL in 10% DMSO.

STORAGE
Vials are to be stored in liquid N₂.

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
HEK cells expressing hGABA\(\alpha_4/\beta_3/\gamma_2\) channels were characterized in terms of their pharmacological properties using whole-cell PatchXpress\textsuperscript{®} and IonWorks\textsuperscript{TM} automated patch clamp platforms.

The cell line was shown to be selectively expressing hGABA\(\alpha_4/\beta_3/\gamma_2\) currents since large inward currents were seen upon exposure to GABA in the extracellular solution in cells held at -40 mV. These currents were blocked by bicuculline, a specific GABA\(\alpha\) channel antagonist. In addition, an EC\(_{20}\) application of GABA elicited currents that were positively modulated by RO 15-4513, but not by diazepam.

Functional channel expression over time was monitored using IonWorks\textsuperscript{TM} HT. Channel expression is robust over at least 28 passages: 88 % of cells expressed hGABA\(\alpha_4/\beta_3/\gamma_2\) currents at passage 28 giving a mean current amplitude at -40 mV of 1.28 ± 1.6 nA (n=330).

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

INTRODUCTION
Gamma-aminobutyric acid (GABA)-gated ion channels are widely distributed in the mammalian brain and are major mediators of inhibitory synaptic transmission. A typical GABA ion channel has a pentameric structure consisting of 5 protein subunits, often \(\alpha, \beta\) and \(\gamma\) or \(\delta\), combining to form a central ion conducting pore across the cell membrane. In humans there are six genes that encode \(\alpha\) subunits, three that encode \(\beta\), three that encode \(\gamma\), and an additional seven genes that encode other subunits whose function is less-well understood than the \(\alpha, \beta\) and \(\gamma\) subunits. GABA ion channels open and close in response to secretion of GABA from presynaptic terminals.

GABA\(\alpha_4\) channels comprised of \(\alpha_4, \beta \cdot \eta, \gamma_2\) subunits are extrasyntaptic, estimated to represent <5% of all GABA\(\alpha\) receptors located in the brain, and insensitive to benzodiazepines (see Mohler & Rudolph 2004 and references therein). Research interest has focused on \(\alpha_4\)-containing receptors because they are potentiated by concentrations of ethanol experienced by human drinkers (1-3 mM); one study showed this effect requires the \(\delta\) subunit is part of the complex (Sundstrom-Poromaa et al., 2002).
FUNCTIONAL VALIDATION

Stability, current amplitude & seal resistance of the PreciSOION hGABA\textsubscript{A} \(\alpha4/\beta3/\gamma2\)-HEK Recombinant Cell Line:

IonWorks\textsuperscript{TM} Electrophysiology.

The cell line was shown to be selectively expressing hGABA\textsubscript{A} \(\alpha4/\beta3/\gamma2\) currents since large inward currents were seen upon exposure to GABA in the extracellular solution in cells held at -40 mV. These currents were blocked by bicuculline, a specific GABA\textsubscript{A} channel antagonist.

**Figure 1. Stability of expression and current amplitude.**
The blue line shows the percentage of cells expressing a mean peak inward current >0.25 nA at -40 mV at cell passages 11, 15, 20, 25 and 28. The red bars show the mean current amplitude (mean ± SD) for 73-330 cells per experiment.

**Figure 2. Seal resistance.**
At passage 28, 97.7% of the cells sealed, and had an average seal resistance of 165.1 ± 46.3 MΩ. The blue line shows the seal rate at passages 11, 15, 20, 25 and 28. The red bars show the mean seal resistance (mean ± SD) for 73-375 cells per experiment.
Figure 3. Frequency distribution of current amplitude obtained in HT mode.
The GABA_α4/β3/γ2 current was defined as the peak current post GABA (100 µM) addition minus the peak current pre GABA addition with a minimum acceptable current of 200 pA. Consistent with published data (4), the majority of cells express current ≤ 1.0 nA.

PatchXpress® Electrophysiology.

Figure 4. GABA concentration-response curve for hGABA_α4/β3/γ2-HEK cells at passage 25 using PatchXpress®. Each point indicates the mean (±) response for 5 cells. We obtained an EC_{50} of 0.840 ± 0.616 µM and a Hill slope of 1.57.
Pharmacology - Bicuculline:

Figure 5. Bicuculline inhibition of response to addition of the EC\textsubscript{80} concentration of GABA. Each point indicates the response of 5 cells. We obtained an IC\textsubscript{50} of 1.44 µM and a Hill slope of -0.775.

Pharmacology – Benzodiazepine site allosteric modulator:

Figure 6. Effect of RO 15-4513 on hGABA\(_{\alpha 4/\beta 2/\gamma 2}\) currents. Previous work (Knoflach et al) demonstrated the positive modifier effect of RO 15-4513 on the hGABA\(_{\alpha 4/\beta 2/\gamma 2}\) current. Here the hGABA\(_{\alpha 4/\beta 2/\gamma 2}\) cells were pre-treated with 100 nM of RO 15-4513 followed by an EC\textsubscript{20} concentration of GABA + 100 nM RO 15-4513. The GABA induced current was increased approximately 2.5-fold by 100 nM RO 15-4513.
Figure 7: The benzodiazepine site allosteric modulator diazepam does not modulate the hGABA_A α4/β3/γ2 current.

Previous work (Knoflach et al.) demonstrated that the benzodiazepine site allosteric modulator diazepam had no effect on the hGABA_A α4/β2/γ2 current. Here we compared the effect of 1 µM diazepam on the normalized GABA (EC_{20}) induced current in HEK cells expressing the hGABA_A α4/β3/γ2 current (n=6 cells; red bars) and the hGABA_A α2/β3/γ2 (n=5; blue bars). 1 µM diazepam increased the EC_{20} GABA-induced current in the hGABA_A α2/β3/γ2 cells, but did not show a positive modulating effect on the hGABA_A α4/β3/γ2 current.
Vectors:

Polylinker: CMV-BamHI-α4-NotI-IRES-neo

hGABA$A_α4$ Sequence:
The sequence of the cDNA clone used for hGABA$A_α4$ corresponds exactly to NM_000809.3
Polylinker: CMV-BamHI-BstXI-β3-NotI-EcoRI-IRES-hyg

hGABA_β3 Sequence:
The sequence of the cDNA clone used for hGABA_β3 corresponds exactly to NM_000814.4.
Polylinker: CMV-NotI-γ2-NotI-EcoRI-BamHI-BstXI-IRES-puro

hGABA\textsubscript{A} γ 3 Sequence:
The sequence of the cDNA clone used for hGABA\textsubscript{A} γ2 corresponds exactly to NM_000816.3.
REFERENCES


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