PRODUCT DATASHEET

PrecisION™ hnAChR α7/ric3-HEK Recombinant Cell Line

Catalog Number: CYL3097

PRODUCT DESCRIPTION
Recombinant HEK cell line expressing the human nAChR α7 ion channel subunit and the chaperone ric3 (Accession numbers NM_000746.4 and NM_024557.3 respectively).

ASSOCIATED PRODUCTS
The PrecisION™ hnAChR α7/ric3-HEK Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:

- CYL3097SS: PrecisION™ hnAChR α7/ric3-HEK Single Site License
- CYL3097TS: PrecisION™ hnAChR α7/ric3-HEK Two Site License
- CYL3097MS: PrecisION™ hnAChR α7/ric3-HEK Multiple Site License

CONTENTS
2 x 1 mL aliquots containing 3.0 x 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 des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneetisesti 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 hnAChR α7/ric3 channels were characterized in terms of their pharmacological properties using whole-cell PatchXpress®, Port-a-Patch® and FLIPR®TETRA™ instruments. The cell line was shown to be selectively expressing hnAChR α7 currents since inward currents were seen upon exposure to acetylcholine and epibatidine in the extracellular solution in cells held at -40 mV. These currents were blocked by methyllycaconitine, a specific hnAChR α7 channel antagonist. Functional channel expression over time was monitored using the PatchXpress® platform. Channel expression is robust over at least 28 passages as >90% of the cells expressed hnAch7 currents >4 nA at passage 28 in the presence of acetylcholine and PNU-120596.
RECOMMENDED CULTURE CONDITIONS

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

INTRODUCTION

The α7 nicotinic acetylcholine receptor (nAChR α7) was originally cloned by Seguela (1) in 1993. These receptors are predominantly expressed in the CNS, including the hippocampus, cortex and ventral tegmental area (2,3,4). nAChR α7 receptors are involved with LTP, memory and attention deficits and so are drug development targets for diseases of cognition including Alzheimer’s and schizophrenia (5). The nAChR α7 receptors are rapidly desensitized, have homopentameric architecture and respond to agonists including nicotine, acetylcholine, AR-R17779 and epibatidine. In addition the currents are blocked by the competitive antagonist methyllycaconitine (6). Drug discovery efforts have also identified positive allosteric modulators such as PNU-120596 (7) which increase the potency of activators such as acetylcholine without causing desensitization. This cell line also expresses the ric3 chaperone protein as when co-expressed with the nAChR α7 receptor, ric-3 significantly increases surface expression (8).

FUNCTIONAL VALIDATION

Stability, current amplitude & seal resistance of the PrecisIOn hνAChR α7/ric3–HEK Cell Line:

PatchXpress® Electrophysiology.

The cell line was shown to be selectively expressing hνAChR α7/ric3 currents since large inward currents were seen upon exposure to acetylcholine and the positive allosteric modulator PNU-120596 (7) in the extracellular solution in cells held at -60 mV. These currents were blocked by methyllycaconitine, a selective nAChR α7 channel antagonist.

Figure 1. Stability of expression and seal resistance

The stability studies on PatchXpress® were determined by preincubating the cells with 5 µM PNU-120596 for 3 minutes followed by the addition of 300 µM acetylcholine + 5 µM PNU-120596 at a holding potential of -60 mV. The blue line shows the percentage of cells expressing a mean peak inward current >4 nA at cell passages 13, 17, 21, 25, 28 and 30. The red bars show the mean seal resistance (mean ± SD) for 8-14 cells per experiment. Functional expression of the hnAChR α7//ric3 currents was > 91% through 30 passages and the mean seal resistance was > 575 MΩ.
Figure 2. The partial allosteric modulator PNU-120595 increases the current activated by the addition of 300 µM acetylcholine in a concentration-dependent manner. The peak inward current was approximately 6 nA and we found an EC_{50} of 926 nM and a Hill slope of 2.73.

Figure 3: The red trace from the PatchXpress® shows approximately 800 pA of current induced by the application of 300 µM acetylcholine alone. The black trace shows the same cell after washout of the acetylcholine followed by a 3 minute preincubation with 5 µM PNU-120596 and then stimulation of the current by the co-application of 5 µM PNU-120596 + 300 µM acetylcholine. There was approximately 8.5 nA of a persistent inward current induced by the co-application of 5 µM PNU-120596 + 300 µM acetylcholine.
**Figure 4.** Currents induced by 300 µM acetylcholine (AC) and 5 µM PNU-120596 (PNU) (red bars) were blocked by the selective nAChR α7 blocker 10 µM methyllycaconitine (MLA) (blue bars).

**Figure 5.** In addition to acetylcholine, several additional agonists can activate the nAChR α7 receptor. The first figure shows the response to epibatidine. Here the cells were treated with 100 µM epibatidine and the current recorded on the PatchXpress. The cell responded with an inward current of approximately 700 pA. The second figure shows the response to AR-R 17779 (9). Here this cell responded to a challenge with 5 µM AR-R 17779 with an inward current of approximately 950pA.
**Port-a-Patch® Electrophysiology.**

**Figure 6.** Currents on the Port-A-Patch®

To examine the reproducibility of currents elicited by repeated application of agonists or modulators, hνAChR α7/ric3-HEK cells were held at -80 mV, and currents elicited by application of 300 µM acetylcholine with 5 µM PNU-120596 were elicited every 2 minutes. Each current trace in Figure 6 was elicited by adding 5 µM PNU-120596 for 500 ms, then adding 300 µM acetylcholine with 5 µM PNU-120596 for 100 ms, then washing with external solution for 6 seconds to allow the current to recover toward the baseline. The wash with external solution continued for an additional minute, then PNU-120596 was added for 30 seconds before eliciting the next current.

Traces one and 18 are superimposed in the figure, and the red arrow points to trace 18.
**Figure 7.** The peak inward current amplitude, and the current integral, recorded for 38 minutes in the whole-cell configuration are shown in the green and blue symbols, respectively.
Figure 8. Cells were pre-loaded with the calcium indicator dye Fluo-8 (Alexa) at 30°C for 90 minutes. Following two wash cycles the cells were placed onto the instrument and the test compounds were added for a 520 seconds preincubation at the following final concentrations: methyllycaconitine = 10µM; PNU120-596 = 5µM. Nicotine was added at 520 seconds at final concentration of 100µM and data collected as graphed below. The external buffer was used as described in (7). The addition of nicotine alone (blue trace) either did not stimulate calcium influx or it was not detectable under these conditions. Preincubation with the positive allosteric modulator PNU-120596 followed by nicotine addition (maroon trace) did result in a large, sustained influx of calcium. This calcium flux was blocked by preincubation with the selective nAChR α7 blocker methyllycaconitine (red trace).
Vector:

Polylinker: CMV-BamHI-NotI-hnAch7-NotI-EcoRI-IRES-neo

The sequence of the cDNA clone used for hnAChR α7 corresponds exactly to NM_000746.4.
Polylinker: CMV- NotI-hric3-EcoRI-IRES- PuroR

The sequence of the cDNA clone used for hric3 corresponds exactly to NM_024557.3
REFERENCES


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