

Human Cortical GABAergic Neurons 2.0 (iPSC-derived)

SKU: 40HU-022

PRODUCT SHEET

Product Description

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system (CNS) and participates in 40% of the inhibitory synapses of adult vertebrates ^[1]. There are several types of GABAergic neurons in the brain, differing in the areas they are formed and where they migrate after neurogenesis ^[2].

Here, we provide cortical GABAergic interneurons, which mediate neuronal function by interacting with excitatory glutamatergic neurons.

GABA signaling has been involved in a plethora of CNS disorders such as Autism ^[3], Parkinson's Disease ^[4], Huntington's Disease, among others.

Product Details

Catalog Number: 40HU-022

Organism: *Homo Sapiens*, Human

Cell Type: Brain Cell

Tissue: N/A

Disease: N/A

Package Size: 1 x 10⁶ cells/vial and 2 x 10⁶ cells/vial

Passage Number: P0

Growth Properties: Adherent

Associated Media: GABAergic Neuron Culture Medium (MD-0122)

Recovery Supplement (MD-0110)

Storage Conditions & Shipment

Product Format/Shipped: Cryopreserved

Storage: Liquid Nitrogen

For Research Use Only

iXCells Biotechnologies USA, Inc.

United States

www.ixcellsbiotech.com

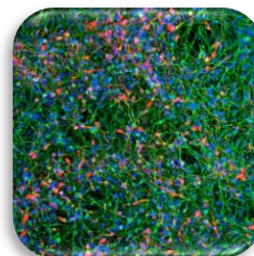
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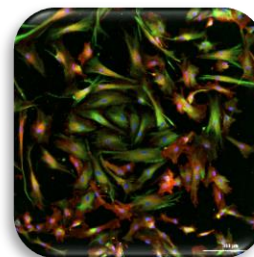
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RELATED PRODUCTS



Human Dopaminergic Neurons
(iPSC-derived, Normal)

SKU: 40HU-002



Human Astrocytes (iPSC-derived,
Normal)

SKU: 40HU-008

Overview of Human Cortical GABAergic Neurons 2.0 (iPSC-derived)

iXCells Biotechnologies is proud to provide fully differentiated and functional **Human Cortical GABAergic Neurons 2.0 (iPSC-derived)** which display typical neuronal morphology (Figure 1), and express typical markers of GABAergic neurons, e.g. GAD67, vGAT (Figure 2, 3), when cultured in the GABAergic Neuron Culture Medium (Cat# MD-0122-100ML). Gamma-aminobutyric acid (GABA) release could be measured by ELISA after 7 days maturation (Figure 4). Moreover, Human GABAergic Neurons 2.0 present spontaneous neuron activity when co-cultured with astrocytes (Figure 5), as assessed by Multi-Electrode Array (MEA), and this activity increases with maturation.

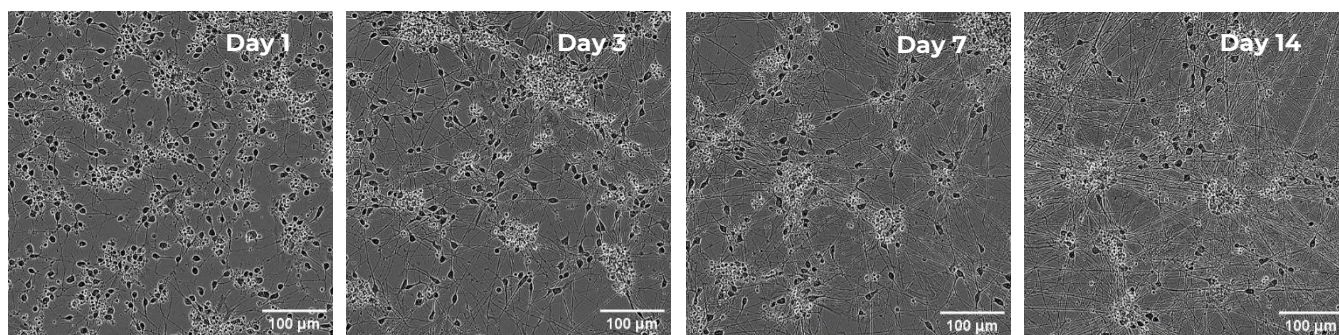


Figure 1. Cells were seeded in Poly-D-lysine/Laminin coated plates at a density of 150,000 neurons/cm². Representative bright field images of GABAergic neurons 2.0 at different time points after thawing. Scale bar: 100µm.

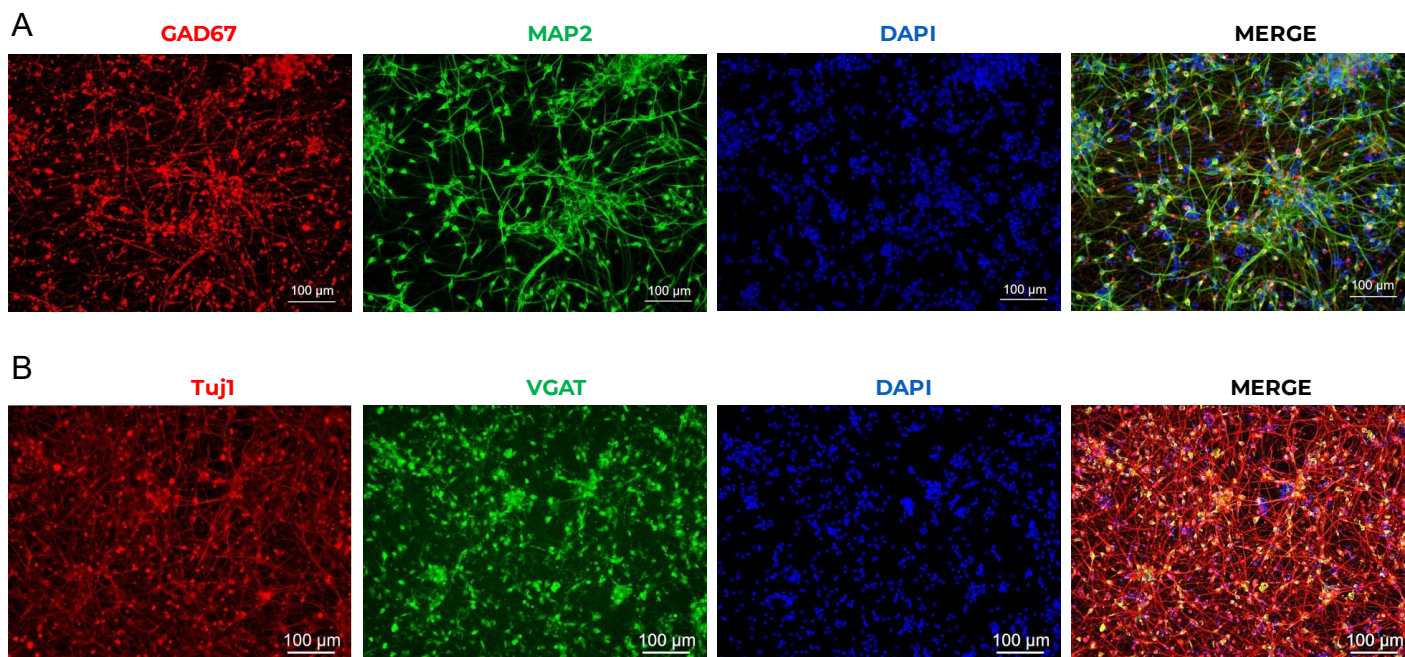


Figure 2. Cells were recovered and seeded in Poly-D-lysine/Laminin coated plates at a density of 150,000 neurons/cm² for 7 days. Immunofluorescence staining showing GAD67/MAP2 (A) and VGAT/Tuji1 (B) positive cells on day 7 in culture. DAPI staining (blue) is also shown. Scale bar: 100µm.

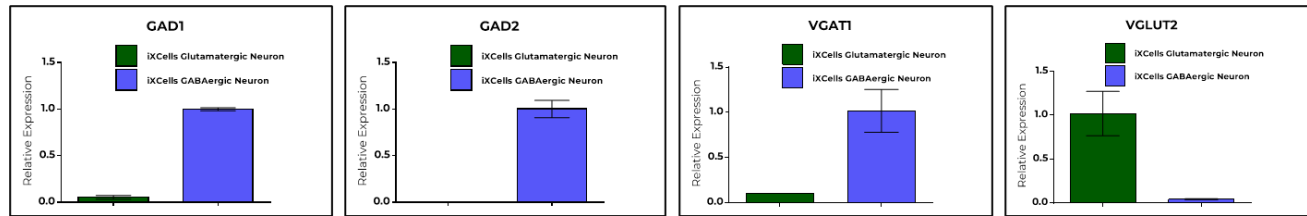


Figure 3. Quantification of RNA expression of the indicated genes by quantitative PCR. Cells were seeded at a density of 150,000 cells/cm² in Poly-D-Lysine/Laminin coated plates and cultured for 14 days post-thawing, then harvested and RNA was extracted for quantification. GAD1, GAD2 and vGAT1 are GABAergic neuron marker genes, and vGLUT2 is a glutamatergic neuron marker gene.

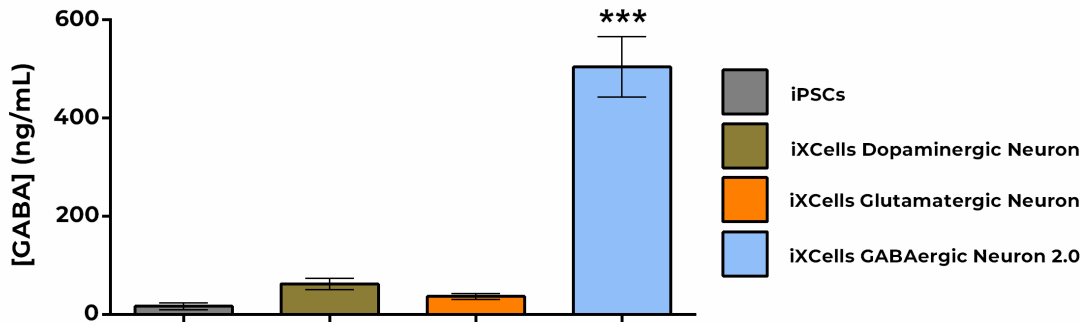


Figure 4. Quantification of GABA in cell culture media by ELISA (Immusmol, Cat#BA-E-2500). Cells were seeded at a density of 100,000 cells/cm² in Poly-D-Lysine/Laminin coated plates and cultured for 7 days. Media samples were collected after 72h of culture post the last media change. GABA ELISA was performed following manufacturer's instructions. One-way ANOVA was used to determine statistical significance (***) against iPSC control.

Representative Raster plot at 30 Days

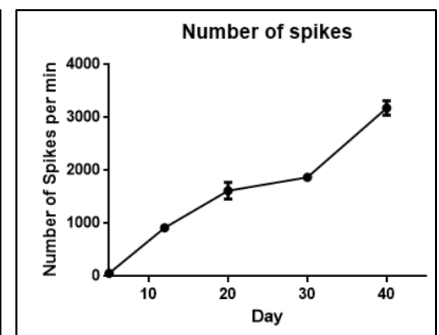
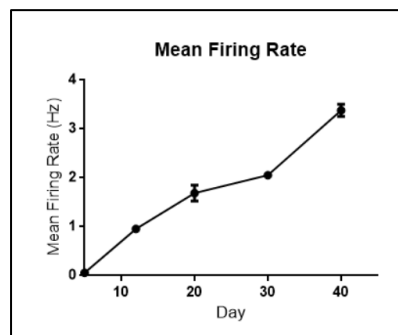
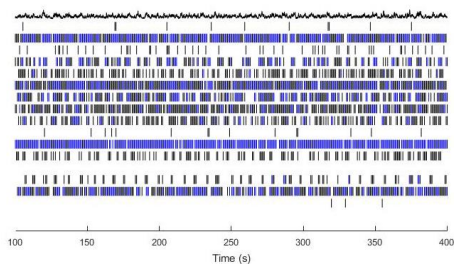


Figure 5. Quantification of Multi-Electrode-Array (MEA) metrics. Cells were co-cultured with astrocytes in 24-well MEA plates, at a density of 100,000 neurons/well and 50,000 astrocytes/well in Polyethyleneimine/Laminin coating. A raster plot showing neuronal network activity and burst for each electrode is shown on the left panel. Mean firing rate quantification is shown on middle panel. Total number of spikes quantification is shown on right panel. Data generated using Axion Biosystems analysis software.

NOTE: CELLS MUST BE CULTURED IN A 37°C, 5% CO₂ INCUBATOR. CELLS ARE ONLY WARRANTED IF IXCELLS MEDIA AND REAGENTS ARE USED AND THE RECOMMENDED PROTOCOLS ARE FOLLOWED.

This procedure describes the steps required for thawing and plating hiPSC-Derived Cortical GABAergic Neurons 2.0 using iXCells™ GABAergic Neuron Culture Medium Kit (Cat# MD-0122).

Mono-culture of hiPSC-Derived Cortical GABAergic Neurons 2.0

The following protocol is based on 48-well plate format.

1. Prepare the coating vessel before thawing the cells. We recommend coating with Poly-D-Lysine (PDL) and laminin. Incubate Poly-D-Lysine solution (1.0 mg/mL; Sigma-Aldrich, Cat# A-003-E) overnight inside of a Biosafety Cabinet (BSC). Wash twice with water the next day, and add the working Laminin solution (10 µg/mL; Sigma-Aldrich, Cat# L2020) into each well to cover the surface. Leave the plate undisturbed at room temperature for at least 2 hours or 1 hour in 37°C CO₂ incubator before use. Aspirate the Laminin solution just before seeding the cells.

Note: Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately to retain the highest cell viability.

2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1-2 minutes. Keep the cap out of water to minimize the risk of contamination.
3. Prepare a mix of **GABAergic Neuron Culture Medium (Cat# MD-0122)** with **Recovery Supplement (Cat# MD-0110-20µL; 1:1000 dilution)** for plating. e.g. for 10 mL GABAergic Neuron Culture Medium add 10 µL Recovery Supplement.

Note: Please spin down the vial of Recovery Supplement before opening it.

4. Pipette the cells into a 15 mL conical tube with 5 mL GABAergic Neuron Culture Medium.
5. Centrifuge at 600 g for 5 minutes at room temperature.
6. Remove the supernatant and re-suspend the cells in GABAergic Neuron Culture Medium supplemented with Recovery Supplement.
7. Seed the cells on precoated plate at the desired density. Incubate in 37°C CO₂ incubator overnight. Perform **complete medium change** the next day with the GABAergic Neuron Culture Medium **without** Recovery supplement.

Note: It is highly recommended to use the pipette when performing complete media change instead of aspirating to avoid disturbing the cells.

Note: It is recommended to seed cells at 100-300K per cm² depending on the applications. Cell debris may be observed after cell recovery because the cryopreserved neurons are fragile. Please refer to the CoA of each lot to determine the seeding density for the experiment.

8. Perform **half medium change** every 2-3 days with GABAergic Neuron Culture Medium without Recovery Supplement. Most of the cells should express high levels of vGAT1 and GAD67 1-2 days after thaw, and express high levels of parvalbumin and MAP2 7-10 days after thaw.

Co-culture of iPSC-Derived Cortical GABAergic Neurons 2.0 with Astrocytes for Multi-Electrode Array assays

The following protocol is based on 24-well plate format.

1. Thaw a vial of iPSC-derived Cortical GABAergic Neuron as described previously, in GABAergic Neuron Culture Medium with Recovery Supplement. Seed the cells on Polyethyleneimine (PEI) and laminin precoated plate at a density of 100,000 cells/well. Add the Polyethyleneimine (PEI) solution (0.07% PEI in 1X Borate buffer), then incubate the plate for 1 hour in 37°C CO₂ incubator. Wash each coated well twice with PBS, and then add sterile water. Aspirate the sterile water and leave the plate inside a BSC overnight. The next day, add the working Laminin solution (10 µg/mL; Sigma-Aldrich, Cat# L2020) into each well to cover the surface. Leave the plate undisturbed at room temperature for at least 2 hours or 1 hour in 37°C CO₂ incubator before use. Aspirate the Laminin solution just before seeding the cells. Incubate in 37°C CO₂ incubator overnight.
2. Perform **complete medium change** the next day with the GABAergic Neuron Culture Medium without Recovery supplement.

Note: It is highly recommended to use the pipette when performing complete media change instead of aspirating.

3. Thaw a vial of Astrocyte (Cat# 40HU-008, check iPSC-derived Astrocyte product page for recovery details) and seed the cells on top of the GABAergic neurons at 5x10⁴ cells per well (24-well plate format), in **GABAergic Neuron Culture Medium without Recovery Supplement** supplemented 1% FBS. We recommend adding astrocytes 1 to 7 days post plating the iPSC derived GABAergic neurons.
4. Incubate in 37°C CO₂ incubator overnight.

5. Perform half medium change every 2-3 days with GABAergic Neuron Culture Medium without Recovery Supplement supplemented 1% FBS (the FBS is used to support the astrocytes).

Note: Penicillin-Streptomycin, Antibiotics and antimycotics, or other antibiotics can be optional in the culture media. iXCells in house test was performed under antibiotics free condition for ≥ 3 days by following the recommended protocol, and no bacterial or fungi contamination were observed during the culture period.

References

- [1] Nicholas CR, Chen J, Tang Y, et al. Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell*. 2013.
- [2] Huang ZJ, Paul A. The diversity of GABAergic neurons and neural communication elements. *Nat Rev Neurosci*. 2019.
- [3] Zhao H, Mao X, Zhu C, et al. GABAergic System Dysfunction in Autism Spectrum Disorders. *Front Cell Dev Biol*. 2022.
- [4] Murueta-Goyena A, Andikoetxea A, Gómez-Esteban JC, Gabilondo I. Contribution of the GABAergic System to Non-Motor Manifestations in Premotor and Early Stages of Parkinson's Disease. *Front Pharmacol*. 2019.

Disclaimers

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