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Human Dopaminergic Neurons (iPSC-derived)

Catalog Number	40HU-002 40HU-002-CHCHD2-R145Q-ISO 40HU-002-CHCHD2-R145Q-HOM	**Cell Number**	1.0 million cells/vial 2.0 million cells/vial
Species	*Homo sapiens*	**Storage Temperature**	Liquid nitrogen

Product Description

Midbrain dopamine (DA) neurons are critical for directing fundamental brain functions such voluntary movement, reward processing, and working memory. The substantia nigra (SN) and the ventral tegmental area (VTA) have the highest populations of DA neurons in the midbrain. The degeneration of DA neurons within the pars compacta region of the SN is a pathological hallmark of Parkinson's disease (PD) and Lewy body dementia (LBD)[1]. For many years, powerful experimental model organisms like the mouse, fruit fly, and baker's yeast have been used to study neurodegenerative diseases, providing insights into disease mechanisms like pathological aggregation of key proteins, the nature and processes of neuronal damage, the role of genetic determinants, and the contribution of neuroinflammation in fueling neuronal loss [2,3]. However, it appears that the use of these models has only partially elucidated some elements of the illnesses, impeding a meaningful translation into new treatments, diagnostics, and prevention.

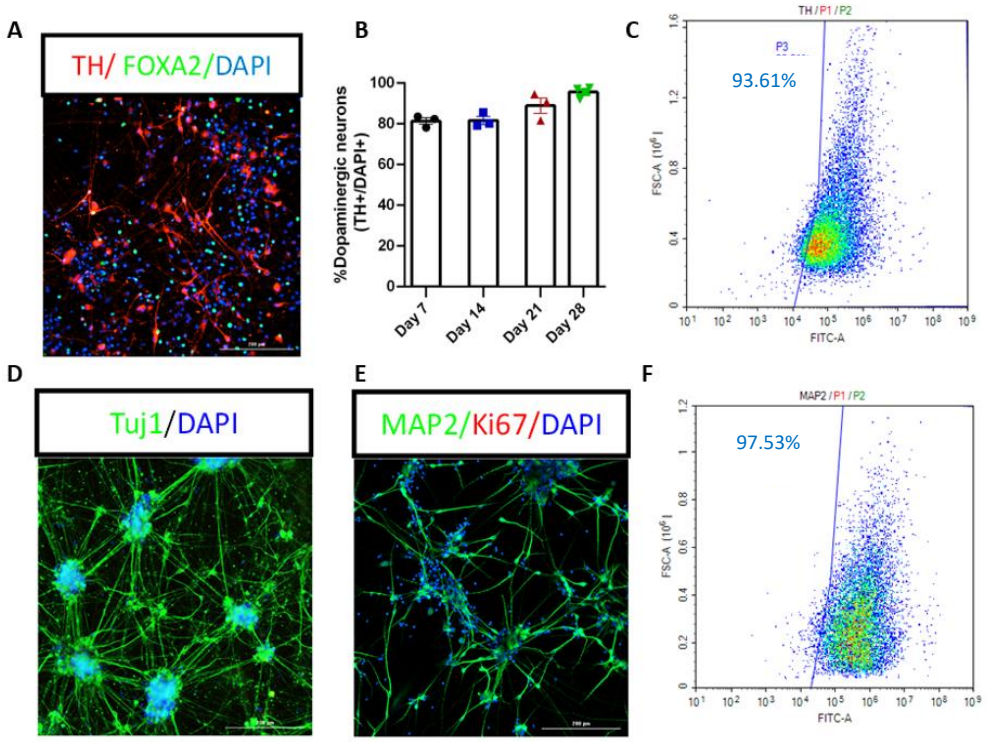

Figure 1. Human iPSCs-derived dopaminergic neurons show expression of characteristic biological markers. **(A)** Immunostaining shows the expression of midbrain dopaminergic neuron markers FoxA2 and TH, 28 days post-thawing. **(B)** Quantifications for % of dopaminergic neurons, positive for the TH marker over multiple time-points. Results are expressed as means ± SEM. **(C)** Flow cytometry measurements demonstrate a highly specific population of fully differentiated midbrain dopaminergic neuron (TH). **(D)(E)** Immunostaining shows the expression of neuron marker Tuj1 (D) 21 days post-thawing and MAP2 (E) 7 days post-thawing and the absence of the proliferative progenitor marker Ki67. **(F)** Flow cytometry measurements demonstrate a highly pure population of fully differentiated neurons (MAP2). Nuclei were counterstained with DAPI. Scale bars, 200 µm.

iXCells Biotechnologies is proud to provide fully differentiated and functional human iPSC-derived DA neurons that display typical neuronal morphology and express all key markers of DA neurons, e.g., TH, FoxA2 (Figure 1) when cultured in the Human Dopaminergic Neuron Maturation Medium (Cat# MD-0105-100ML). In addition, our iPSC-derived DA neurons can also be co-cultured with glial cells or other cell types for drug screening platforms. Moreover, functional assays revealed that these neurons display calcium transient activity (Figure 2) and whole cell patch clamp revealed that over 75% of the neurons exhibited mature spiking, and over 50% of the neurons had spontaneous activity at a holding potential of -45 mV (Figure 3) indicating the presence of a highly mature population of neurons.

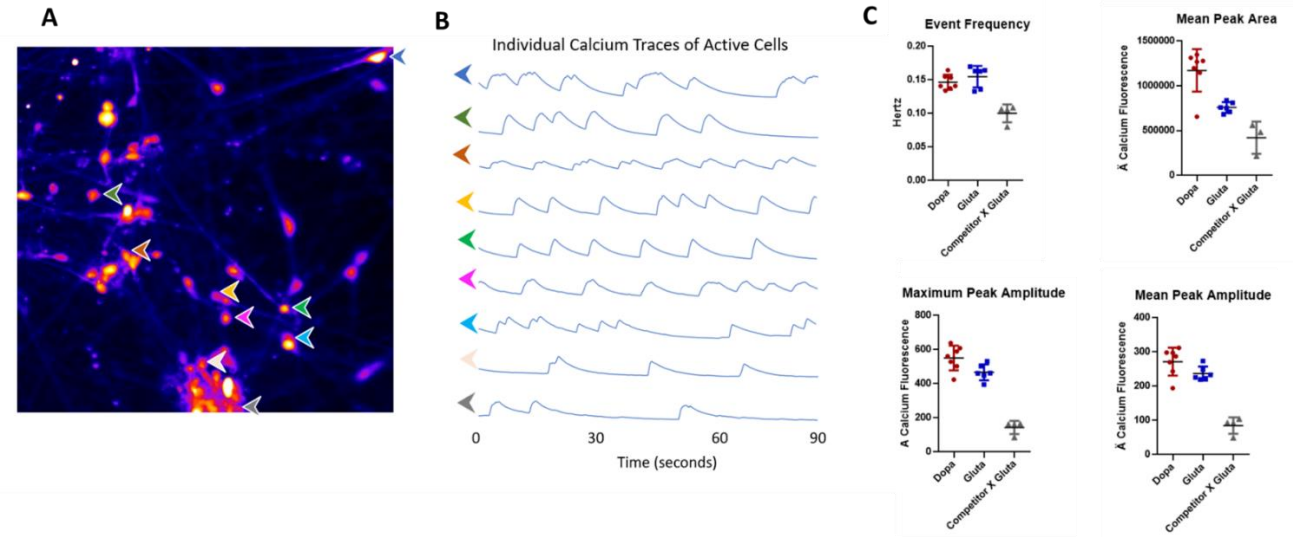

Figure 2. (A) Live single cell calcium activity was recorded using Cal-Bryte-590 fluorescent calcium indicator and imaged on the IC200 KIC® (Kinetic Image Cytometer) (Vala Sciences) and analyzed using CyteSeer® (Vala Sciences). **(B)** Individual Calcium Traces are color coded with respective arrows. **(C)** Event Frequency, maximum and mean peak amplitude, mean peak area are compared between iXCells dopaminergic neurons (red, Cat# 40HU-002), iXCells cortical neurons (blue, Cat# 40HU-009) and competitor X glutamatergic neurons (grey). The results are graphed as mean ± SEM.

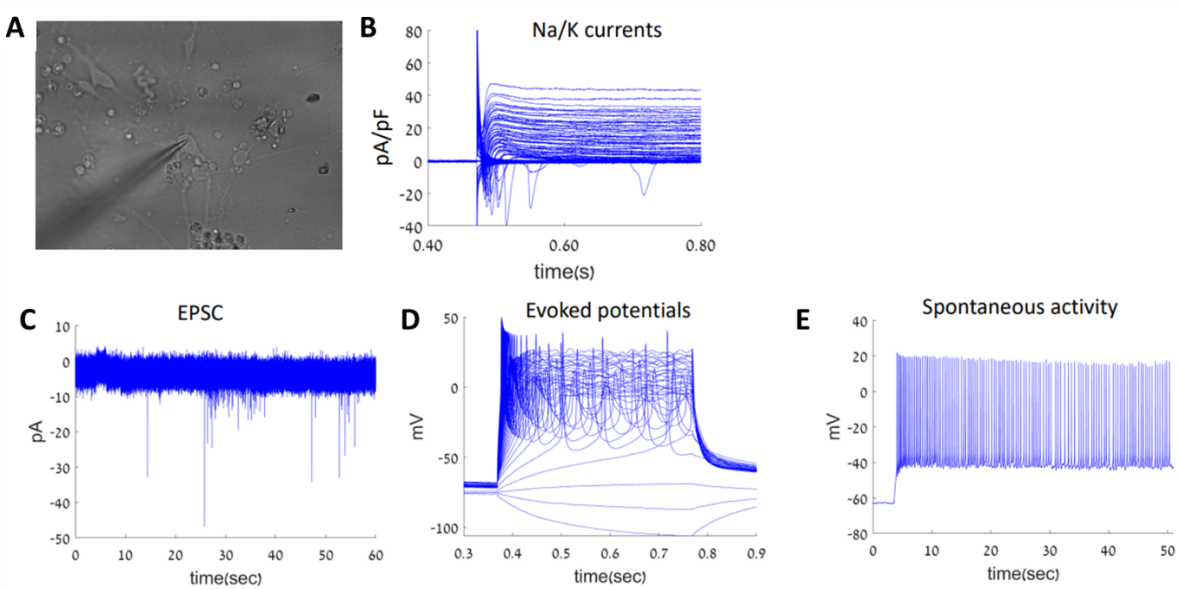

Figure 3. (A) An image of the cells during the experiment. The patch pipette is visible on the left. **(B)** The Sodium/potassium currents were recorded in voltage clamp mode with test potentials of -100 mV to 90 mV. **(C)** Excitatory postsynaptic currents (EPSCs) were recorded in voltage clamp mode while clamping the cell at -60 mV. **(D)** Evoked action potentials were recorded in current clamp mode starting with a current injection 12 pA below what is needed to hold the neuron at - 60 mV and with 3pA current steps. **(E)** Spontaneous activity was recorded in current clamp mode with a current injection needed for a membrane potential of -45 mV.

Product Details

Tissue Origin	Human iPSC-derived dopaminergic neurons
Package Size	1.0 million cells/vial; 2.0 million cells/vial
Shipped	Cryopreserved
Storage	Liquid Nitrogen
Media	Human Dopaminergic Neuron Maturation Medium (Cat# MD-0105-100ML) Recovery Supplement (Cat# MD-0110-20µL)

Protocols

Monoculture of hiPSC-Derived Dopaminergic Neurons

The following protocol is based on 48-well plate format

1. Prepare the coating vessel before thawing the cells. Coated plate preparation: coat the plates with Poly-L-ornithine solution (Sigma-Aldrich; P4957;10 µg/ml) in water overnight in the incubator, wash twice with water the next day and coat the plates with laminin (Sigma-Aldrich; L2020; 5 µg/ml) in PBS overnight. Do not let dry at any stage.

   ***Note**: Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately in order 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. Pipette the cells into a 15 mL conical tube with 5 mL **Human Dopaminergic Neuron Maturation Medium (Cat# MD-0105-100ML).**

4. Centrifuge at 300*g* for 5 minutes at room temperature.

5. In the meantime, calculate the volume of cell suspension needed to plate the neurons and prepare the media by adding the **Recovery Supplement** (**MD-0110-20uL**; 1:1000 dilution) to the Human Dopaminergic Neuron Maturation Medium, e.g. for 5 mL of Human Dopaminergic Neuron Maturation Medium add 5 µL of Recovery Supplement.

6. Remove the supernatant and re-suspend the cells in Human Dopaminergic Neuron Maturation Medium + Recovery Supplement. Recovery supplement can be kept for 4 days -followed by either being removed completely or slowly diluted out (i.e. 50% media change without supplement).

7. Seed the cells on precoated plate at the desired density. Incubate in 37ºC CO_2 incubator overnight.

   ***Note:** We recommend seeding 90-200K cells per cm^2 depending on the application. Cell debris may be observed after cell recovery. Refer to the CoA of each lot to determine the seeding density for your experiment.*

8. Perform half medium change every 2-3 days. Most of the cells should express high levels of Tuj1 and MAP2, 2 days after thaw, and express high levels of TH and FOXA2 7-10 days after thaw.

   ***Note:** DA neurons tend to aggregate and detach from the plates. Change 50% of the medium with extra care to avoid cell loss.*

Co-culture of hiPSC- Derived Dopaminergic Neurons with Astrocytes

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

1. Thaw a vial of iPSC-derived dopaminergic neurons as described previously, in Human Dopaminergic Neuron Maturation Medium (Cat# MD-0105) with Recovery Supplement (Cat# MD-0110; 1 in 1000). Seed the cells on precoated plate at the desired density. Incubate in 37°C CO_2 incubator overnight.
2. Perform ***complete media change*** to remove the Recovery Supplement the next day.

   Note*: We recommend using a 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 dopaminergic neurons at $2x10^4$ cells per well (48-well plate format), in Human Dopaminergic Neuron Maturation Medium with 1% FBS. We recommend adding astrocytes 1 to 7 days post plating the iPSC derived dopaminergic neurons.
4. Incubate in 37°C CO_2 incubator overnight.
5. Perform half medium change every 2-3 days (keeping the 1% FBS to support the astrocytes).

References

[1] Bloem, B. R., Okun, M. S. & Klein, C. (2021) Parkinson's disease. *Lancet* 397, 2284–2303.
[2] Schulz-Schaeffer, W.J. (2015). Is Cell Death Primary or Secondary in the Pathophysiology of Idiopathic PD? *Biomolecules* 5, 1467-1479.
[3] Poewe, W., Seppi, K., Tanner, C. et al. (2017). Parkinson disease. *Nat Rev Dis Primers* 3, 17013

Disclaimers