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Product Information

Human Dopaminergic Neurons (iPSC-derived)

Catalog Number	40HU-012	Cell Number	2.0 million cells/vial (Cryopreserved)
Species	*Homo sapiens*	Storage Temperature	Liquid nitrogen

Product Description

Dopaminergic (DA) neurons are the major cells generating a neurotransmitter, dopamine, in the central nervous system. DA neurons reside in various regions extending from the midbrain to the forebrain in mammals. Loss of DA neurons in the *substantia nigra pars compacta* (SNc; AKA A9 group) in the midbrain region may contribute to the motor disturbance in Parkinson's disease (PD)[1]. However, what causes DA neuron to degenerate or die is still unknow[1]. To understand the PD progression and develop effective therapeutic approaches in laboratories, a clinical relevant and cell-based platform is needed[1,2,3]. Therefore, human induced pluripotent stem cell (iPSC) derived DA neurons can serve as a powerful tool to investigate the underlying molecular mechanisms, to model AD *in vitro* for drug discovery, and to pave the road for cell therapy development[4].

iXCells Biotechnologies provides DA neuron-like cells derived from human iPSCs. Our hiPSC-derived DA neurons express typical DA neuron marker, tyrosine hydroxylase (TH), as well as mature neuron marker microtubule associated protein 2 (MAP2) (**Figure 1**). iXCells DA neurons are available in cryopreserved vials (2 x 10^6 cells/vial). The cells can be recovered in the DA Neuron Maintenance Medium. And after 10-14 days of recovery, these cells will mature into neuron-like cells and will express high levels of TH and dopamine transporter (DAT) (**Figure 1, 2, and 3**).

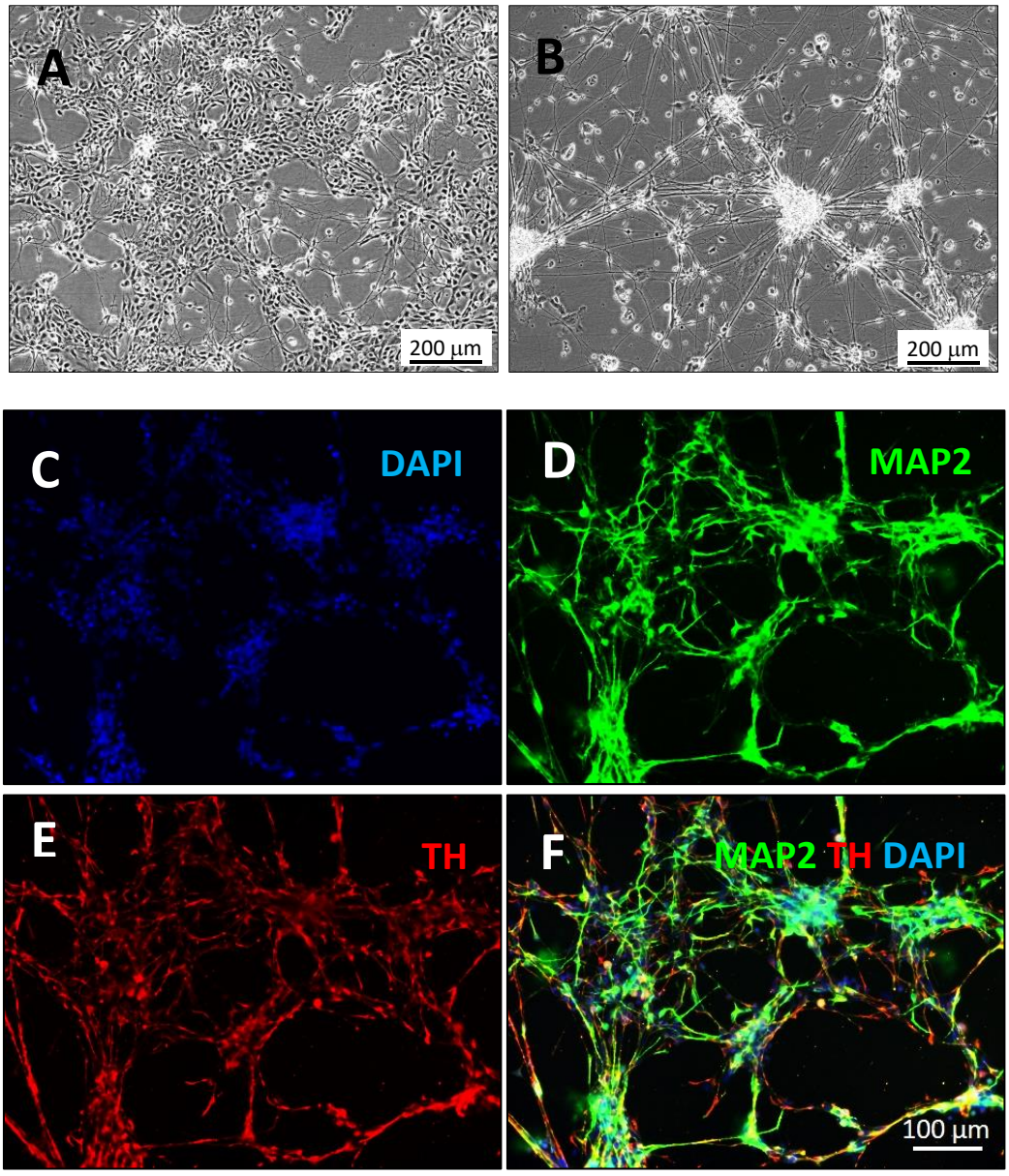

Figure 1. Mature DA neuron-like cells derived from human iPSCs highly express MAP2 and TH markers. (A) Cells were recovered and cultured for 4 days in Human Dopaminergic Neuron Maintenance Medium (Cat# MD-0105A). Robust neurogenesis was observed. **(B)** Cells were further cultured and matured in Human Dopaminergic Neuron Maturation Medium (Cat# MD-0105B). **(C)** to **(F)** Representative images show that DA neurons express MAP2 and TH. Cells were recovered from cryovial and cultured for 11 days. Identity of DA neurons was confirmed by immunostaining using MAP2 and TH antibodies. More than 85% of the human iPSC-derived DA neurons are TH positive (estimated by immunostaining). Nuclei were stained with DAPI.

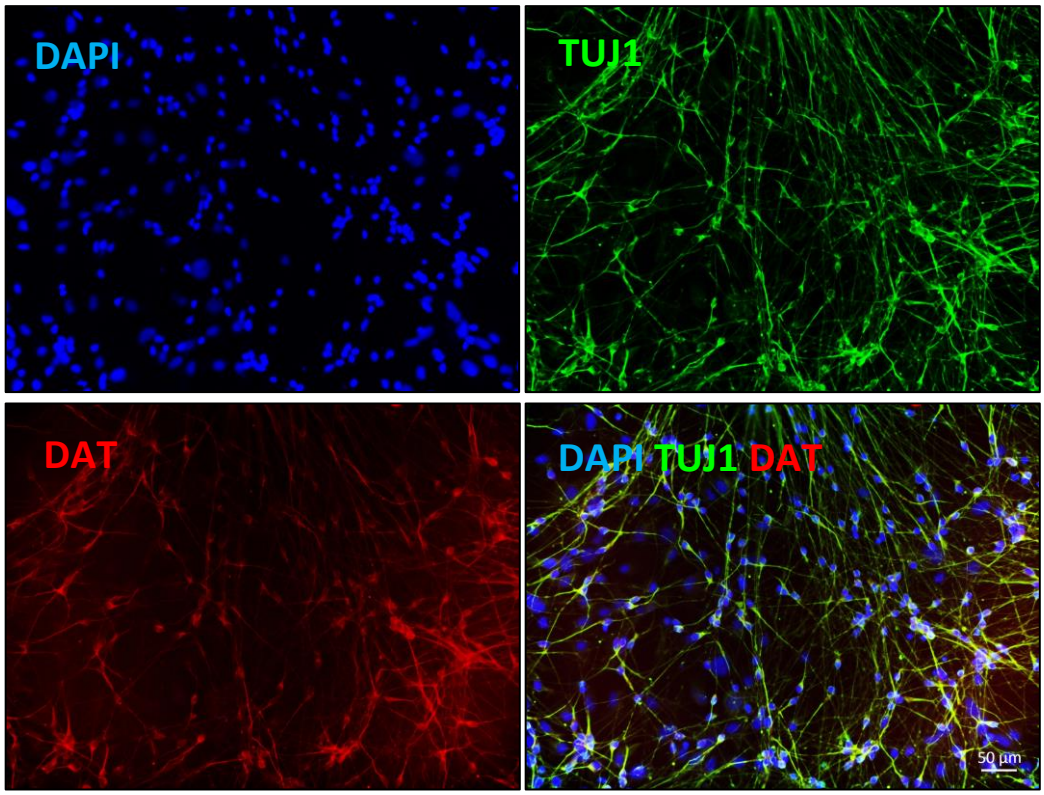

Figure 2. Many TUJ1+ neuron-like cells express DAT. Cells were cultured for 2 weeks and used to perform immunostaining of TUJ1 and DAT. More than 50% of the human iPSC-derived DA neurons are DAT positive (estimated by immunostaining). Nuclei were stained with DAPI.

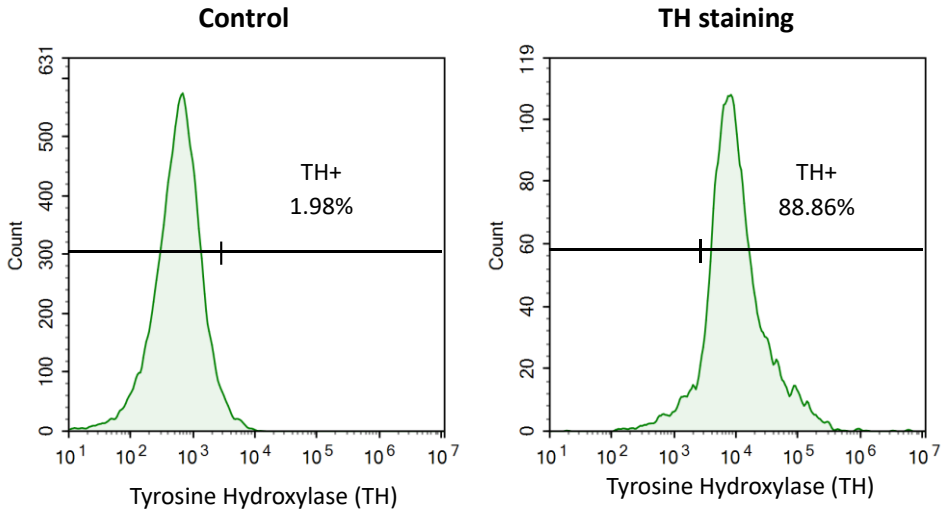

Figure 3. Majority of mature neurons express tyrosine hydroxylase. Cells were cultured for 2 weeks and used to perform immunostaining of TH. TH signal was detected and quantified by flow cytometry analysis. More than 80% of the human iPSC-derived DA neurons are TH positive. Non-staining cells serve as negative control (Control).

Product Details

Tissue Origin	Human iPSC-derived Dopaminergic Neurons (Normal)
Package Size	2 x 10^6 cells/vial (frozen) The kit contains 20ml of Human Dopaminergic Neuron Recovery Medium (Cat# MD-0105A)
Shipped	Cryopreserved
Media	Human Dopaminergic Neuron Recovery Medium (Cat# MD-0105A) Human Dopaminergic Neuron Maturation Medium (Cat# MD-0105B)

Protocols

Recovery and Maturation of hiPSC-Derived Dopaminergic Neurons

1. 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. Prepare plates coated with Coating Buffer I one day before thawing the cryopreserved cells.
3. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the risk of contamination.
4. Pipette the cells into a 15ml conical tube with 4 ml of **Human Dopaminergic Neuron Recovery Medium** (Cat# MD-0105A).
5. Centrifuge the cells at 200 *x g* for 5 minutes at room temperature.
6. Remove the supernatant and re-suspend the cells in **Human Dopaminergic Neuron Recovery Medium** containing 10 μM of ROCK inhibitor (Stem Cell Technologies, Cat# 72302).
7. Seed the cells on coated plates at the desired density.

   Note: We recommend seeding the cells at the density of 50,000 to 100,000 cells/cm^2. The optimal seeding density will need to be determined by end users for different assays and analysis.
8. Grow the seeded cells in 37°C CO_2 humidified incubator overnight.
9. Perform half medium change every 2-3 days.

   Note: Neurogenesis should start in the first week. Neuron-like cell morphology will appear. Neurons tend to aggregate and detach from the plates.
10. When robust neurogenesis is observed (~4 to 7 days after recovery) as shown in Figure 1A, change 100% of the spent medium to **Human Dopaminergic Neuron Maturation Medium** (Cat# MD-0105B). Handle it gently to avoid cell loss.

11. From now on, change 50% of the medium once or twice a week with **Human Dopaminergic Neuron Maturation Medium** according to the cell confluency. Change 50% of the medium with extra care to avoid cell loss.
12. Tyrosine hydroxylase (TH) will be expressed two weeks after recovery. Cells can be further cultured for 2 more weeks or longer.

References

[1] Julie Lotharius and Patrik Brundin. “Pathogenesis of Parkinson’s disease: dopamine, vesicles and a-synuclein” *Nat Rev Neurosci* 2002 3(12):932-42.

[2] Ernest Arenas, Mark Denham, J. Carlos Villaescusa. “How to make a midbrain dopaminergic neuron” *Development* 2015 142:1918-1936.

[3] Rodolfo Gonzalez, et al., “Deriving dopaminergic neurons for clinical use. A practical approach” *Scientific Reports* 2013 3:1463-1467.

[4] Curt R. Reed, et al., “Transplantation of Embryonic Dopamine Neurons for Severe Parkinson's disease” *N Engl J Med* 2001 344:710-719.

Disclaimers