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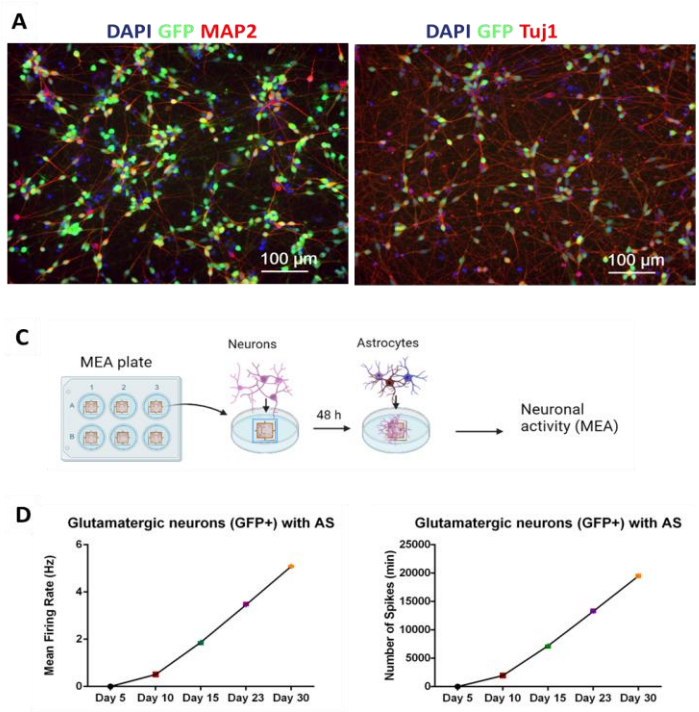
Human Glutamatergic Neurons (iPSC-derived, GFP-labeled, Normal)

Catalog Number	40HU-014-GFP	**Cell Number**	1.0 million cells/vial 2.0 million cells/vial
Species	*Homo sapiens*	**Storage Temperature**	Liquid nitrogen

Product Description

The rapid and highly reproducible generation of mature and functioning neurons from human pluripotent stem cells (hPSCs), neural progenitors, or fibroblasts has been made possible through the utilization of transcription factors. These factors have played a pivotal role in facilitating investigations related to neurodevelopment, disease modeling, drug screening, and neuronal replacement therapies. By employing various combinations of transcription factors along with specific small molecules, researchers have successfully generated populations of glutamatergic neurons [1,2]. This significant breakthrough has provided valuable insights into neural processes and offered potential avenues for therapeutic advancements.

iXCells Biotechnologies takes pride in offering fully differentiated and functional human iPSC-derived glutamatergic neurons that exhibit typical neuronal morphology and express key markers associated with glutamatergic identity, such as VGLUT2, MAP2, and TUJ1 (Figure 1A, B). These neurons not only display robust neural activity but also show an increase in activity over time, indicating progressive maturation (Figure 1C, D). When cultured in the Human Glutamatergic Neuron Maturation Medium (Cat# MD-0116), our neurons provide a reliable model for studying neuronal function and responses (Figure 2). Additionally, our iPSC-derived neurons can be co-cultured with glial cells, enabling the development of comprehensive drug screening platforms to evaluate drug efficacy, neurotoxicity, and other neural responses.



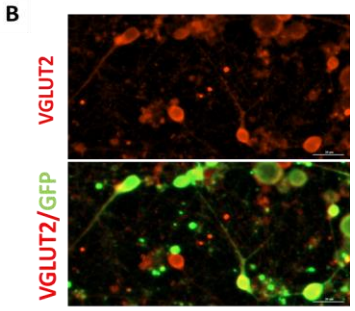


Figure 1. Human iPSCs-derived glutamatergic neurons show expression of characteristic biological markers. **(A)** Immunostaining shows the expression of neuronal makers MAP2 and Tuj1 at 5 days post-thawing. **(B)** Immunostaining shows expression of glutamatergic marker VGLUT2, 25 days post-thawing Scale bar 30µm**. (C)** Glutamatergic neurons were differentiated as a monolayer on a MEA plate and co-cultured with iPSC-derived Astrocytes (40HU-008) and subsequently neuronal activity was measured. **(D)** The electrical parameters were measured in the MEA over time and the results are graphed as mean ± SEM for mean firing rate and number of spikes.

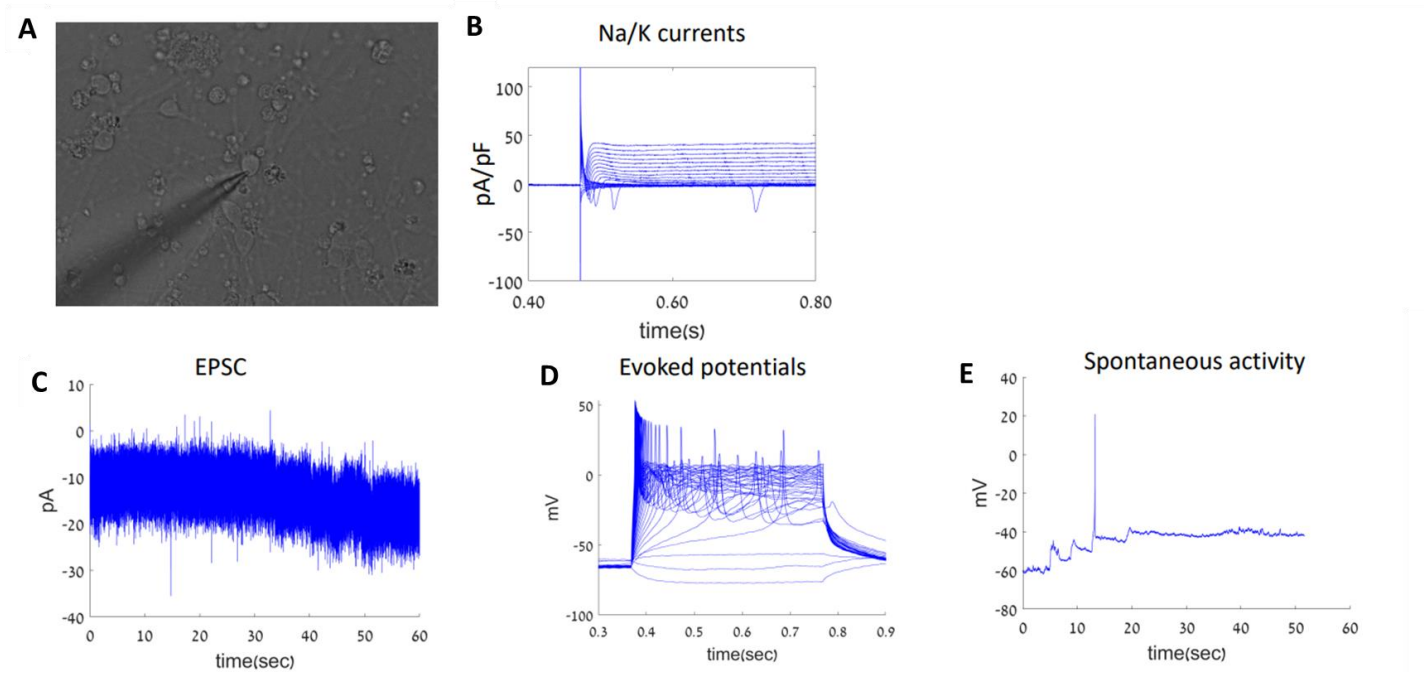


Figure 2. (A) An image of the cells during the experiment. The patch pipette is visible on the left. **(B)** The Sodium/potassium currents were recorded in voltage clamp mode with test potentials of -100 mV to 90 mV. **(C)** Excitatory postsynaptic currents (EPSCs) were recorded in voltage clamp mode 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 Glutamatergic Neurons (Normal)
Package Size	1.0 million cells/vial; 2.0 million cells/vial;
Shipped	Cryopreserved
Storage	Liquid Nitrogen
Media	Human Glutamatergic Neuron Maturation Media (Cat# MD-0116-100ML) Recovery Supplement (Cat# MD-0110-20UL)

Protocols

Mono-culture of hiPSC-Derived Glutamatergic Neurons

The following protocol is based on 12-well plate format

1. Prepare the coating vessel before thawing the cells. Please refer to the iXCell's coating protocol. Double coating, e.g. PDL+ Matrigel is preferred for these cells.

***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 Glutamatergic Neuron Maturation Media (Cat# MD-0116-100ML).**
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; 1:500 dilution) to the Human Glutamatergic Neuron Maturation Media, e.g. for 5 mL of Human Sensory Neuron Maintenance Medium add 10 µL of Recovery Supplement.
6. Remove the supernatant and re-suspend the cells in Human Glutamatergic Neuron Maturation Media + Recovery Supplement. Recovery supplement can be slowly diluted out with the subsequent media changes (i.e. 50% media change without supplement).
7. Seed the cells on precoated plate at the desired density. Incubate in 37°C CO_2 incubator overnight.

***Note:** We recommend seeding 100K-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.*

Perform half medium change every 3-4 days. Most of the cells should express high levels of MAP2 and TuJ1, 5-7 days after thaw.

References

[1] Yingsha Zhang, Changhui Pak, Yan Han, Henrik Ahlenius, Zhenjie Zhang, Soham Chanda, Samuele Marro, Christopher Patzke, Claudio Acuna, Jason Covy, Wei Xu, Nan Yang, Tamas Danko, Lu Chen, Marius Wernig, Thomas C Südhof (2013) "Rapid single-step induction of functional neurons from human pluripotent stem cells''. *Neuron*, 78,785-798

[2] Ralda Nehme, Emanuela Zuccaro, Sulagna Dia Ghosh, Chenchen Li, John L Sherwood, Olli Pietilainen, Lindy E Barrett, Francesco Limone, Kathleen A Worringer, Sravya Kommineni, Ying Zang, Davide Cacchiarelli, Alex Meissner, Rolf Adolfsson, Stephen Haggarty, Jon Madison, Matthias Muller, Paola Arlotta, Zhanyan Fu, Guoping Feng, Kevin Eggan. (2018) "Combining NGN2 programming with developmental patterning generates human excitatory neurons with NMDAR-mediated synaptic transmission". *Cell Rep.,* 23, 2509-2523.

Disclaimers