B-27 Plus Neuronal Culture System for PSC-derived neurons

Human induced pluripotent stem cell (iPSC)–derived neurons have increasingly become a valuable system for the study of neurological disorders. Improved differentiation protocols, cell reprogramming, and gene editing enable scientists to generate patient-specific, disease-in-a-dish models for disorders such as Parkinson's disease, Alzheimer's disease, and autism, among others. These human models tend to be flexible and scalable, and maintain many of the characteristics found in these disorders, which are key requirements for their use in mechanistic and drug discovery studies.

A critical step in generating useful PSC-derived neurons is neuronal maturation. During maturation, neurons extend neurites to form highly connected networks, express synaptic markers, and generate spontaneous, networked electrical activity. Robust maturation is necessary for PSC-derived neurons to be relevant disease model systems. Typical maturation conditions are inefficient, generating poorly matured neurons with low levels of functionality. Recently we developed a neuronal maturation and maintenance medium, the Gibco™ B-27™ Plus Neuronal Culture System, which includes a user guide specifically for PSC-derived neurons. This next-generation system was designed to improve long-term neuronal survival, maturation, and functionality of neurons in culture.

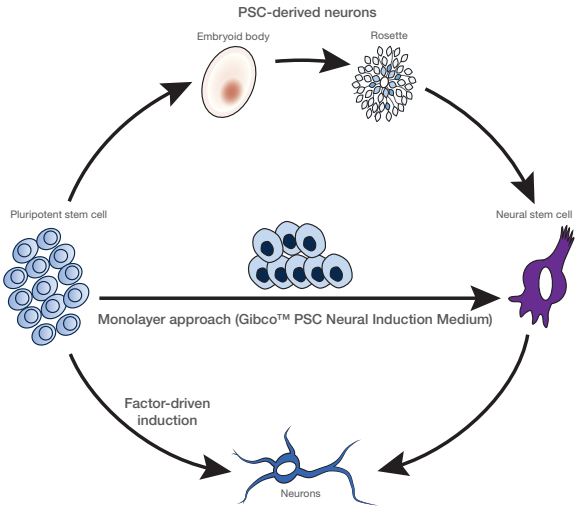

Figure 1. Methods for generating PSC-derived neurons. Schematic of the three most common methods used for generating PSC-derived neurons.

Here we provide guidance and demonstrate the benefits of using the B-27 Plus system with the most frequently used PSC-derived neuron model systems (Figure 1). Key strengths offered by this system:

- Improves neuronal health resulting in enhanced long-term survival (Figure 4 on page 3)
- Accelerated maturation of neurons as seen by increased synaptic marker expression versus classic B-27 Supplement and Gibco™ Neurobasal Medium (B-27 classic system; Figure 5 on page 3)
- Enhances functional maturation resulting in high levels of spontaneous, synchronous network activity compared to B-27 classic system or the BrainPhys™ Neuronal Medium and SM1 Neuronal Supplement (STEMCELL Technologies) (Figures 6 and 7 on page 4)

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Methods for generating PSC-derived neurons

The most commonly used methods for generating PSC-derived neurons include rosette formation, the monolayer approach, and factor-driven induction (Figure 1 on page 1).

The rosette formation and monolayer differentiation methods mimic *in vivo* development of PSC-derived neurons, and both involve an intermediate step that yields an expandable population of neural stem cells (NSCs) that can be further differentiated into neurons. NSC methods are flexible and may be more relevant to disease modeling.

Another method is factor-driven induction of PSCs into neuronal "iN" cells. This method typically involves overexpression of lineage-specific factors to rapidly induce a neuronal cell fate. The benefits of this system are rapid and highly efficient generation of functional neurons within 14 days. Factor-driven induction can also be used to generate induced neurons from somatic cells.

Guidance for switching to the B-27 Plus system during NSC differentiation when using a monolayer or rosette method

When differentiating NSCs that were created using a monolayer or rosette method, we recommend switching to the B-27 Plus system after 3–7 days or when the cell population has adopted a neuronal-like morphology. Figure 2 shows example images of what cells look like in the initial stages of NSC neuronal differentiation (A,B) and demonstrates cells that are ready to transition to B-27 Plus media (C). In this example, the cells have a neuronal-like morphology with neurites extending out and contacting neighboring cells. The specific components of the recommended NSC neuronal differentiation media and further guidance are provided in the B-27 Plus system protocol for PSC-derived neurons.

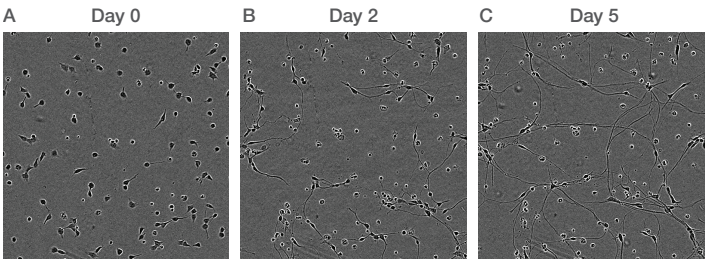


Figure 2. NSC differentiation guidance on switching to the B-27 Plus system.

Guidance for using the B-27 Plus system with factor-driven induced neurons

An example of an induced neuron workflow is outlined in Figure 3. Starting with PSCs, neuronal generation typically involves three steps: delivery of lineage-inducing factor(s) if not selecting for stable expression, regulated induction or expression of factors to initiate neural fate, and neuronal maturation. In this system, we recommend using the B-27 Plus system for the neuronal maturation step and continued maintenance of the induced neurons.

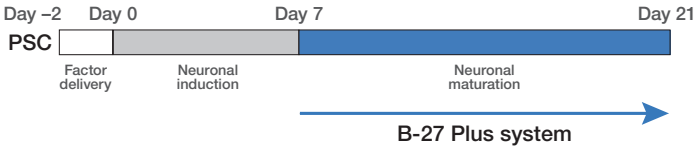


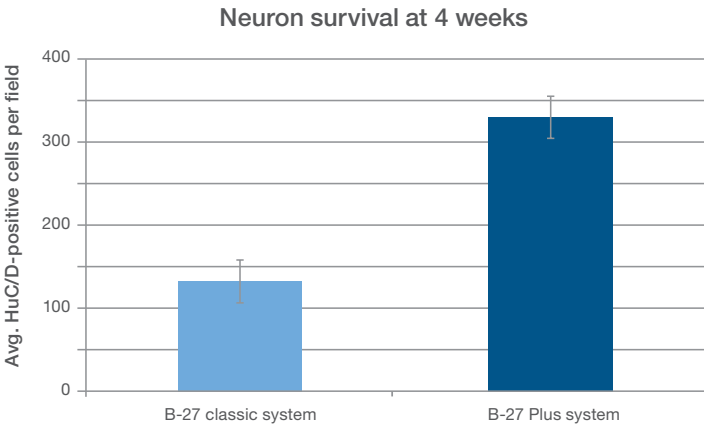
Figure 3. Factor-driven induced neuron workflow. Schematic representation of the factor-driven method. The timelines of each step are approximations as there are many variations of this method.

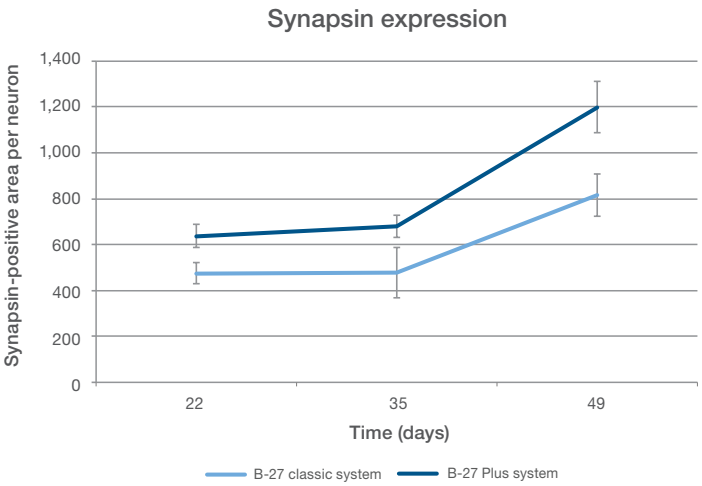
What to expect

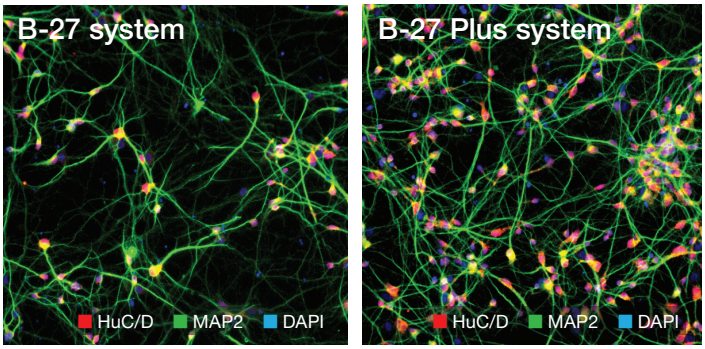
The B-27 Plus Neuronal Culture System was designed to improve survival, maturation, and functionality of neurons. Here we demonstrate the benefits of using the B-27 Plus system in the three different PSC-derived neuron model systems described earlier.

Enhanced survival

Long-term survival of PSC-derived neurons is particularly important for studying human disease systems. Human disease model systems require long time periods before they display particular disease phenotypes. The B-27 Plus system improves neuronal health and survival, enabling long-term culture and maturation of human iPSC (hiPSC)-derived neurons. Figure 4 on page 3 shows hiPSC-derived cortical neurons (from rosette NSCs) matured for four weeks in either the B-27 Plus or B-27 classic system. Cultures matured and maintained in the B-27 Plus system had over twice as many neurons compared to the B-27 classic system.







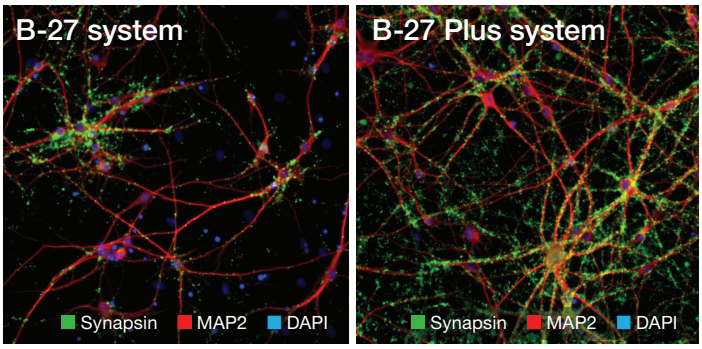


Figure 4. The B-27 Plus system enhances long-term survival of neurons matured from rosette NSCs. Rosette NSC neurons matured in B-27 Plus Supplement for 4 weeks, resulting in an approximately 2-fold increase in survival compared to classic B-27 Supplement and Neurobasal Medium.

Figure 5. The B-27 Plus system increases synaptic marker expression in neurons matured from monolayer NIM NSCs. PSC Neural Induction Medium (NIM) monolayer NSC neurons matured in the B-27 Plus system for 7 weeks results in significantly higher levels of synapsin 1/2 expression versus classic B-27 media system. Synaptic marker expression is an indicator of functional maturity.

Increased synaptic complexity

One way to measure neuronal maturation is to look at expression of synaptic markers. Synapsin is a protein localized to synapses where it is involved in regulating the release of neurotransmitters. Synapsin expression is an indicator of synapse formation and neuronal connectivity in developing neuronal networks. Figure 5 compares synapsin expression in hiPSC-derived cortical neurons (from monolayer NSCs) cultured in the B-27 Plus or B-27 classic media system. Neurons cultured in the B-27 Plus system had approximately 50% higher levels of synapsin expression compared to the B-27 classic system.

Improved functionality

During development, neurons form complex networks through the formation of synaptic connections. As these networks mature, spontaneous neuronal activity synchronizes into temporally consistent network bursts. Synchronous spontaneous network activity is a key indicator of mature neural networks. Impaired network activity is a common feature of neurological diseases such as autism, schizophrenia, and Alzheimer's disease. For PSC-derived neurons to be useful model systems, they must show synchronous spontaneous network activity. Figure 6 on page 4 shows multielectrode array (MEA) data comparing electrophysiological activity of hiPSC-derived neurons (monolayer NSC) cultured in B-27 Plus, B-27 classic, or BrainPhys SM1 culture systems. Neurons cultured in the B-27 Plus system showed significantly higher levels of spontaneous synchronized bursting compared to both B-27 classic and BrainPhys SM1 systems. Figure 7 on page 4 shows MEA data comparing factor-driven induced neurons cultured in either

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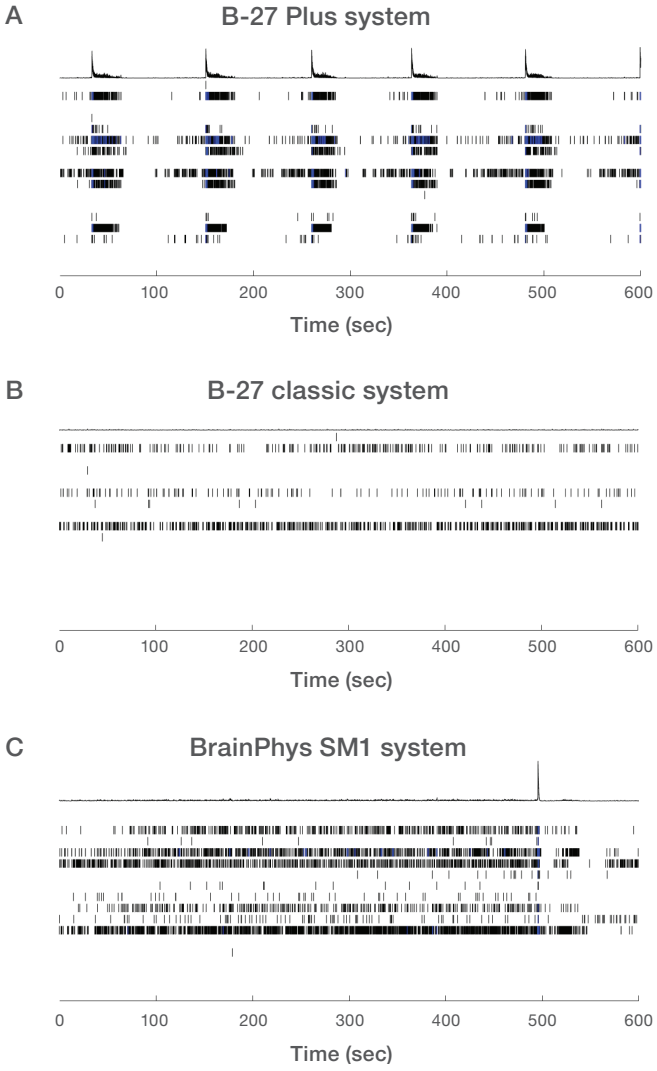

Figure 6. The B-27 Plus system improves functional activity in neurons from monolayer NIM NSCs. PSC Neural Induction Medium (NIM) monolayer NSC neurons matured in B-27 Plus Supplement for 31 days showed high levels of spontaneous synchronous network bursting activity compared to both B-27 classic and the BrainPhys SM1 culture systems. This networked activity was maintained for over 4 weeks.

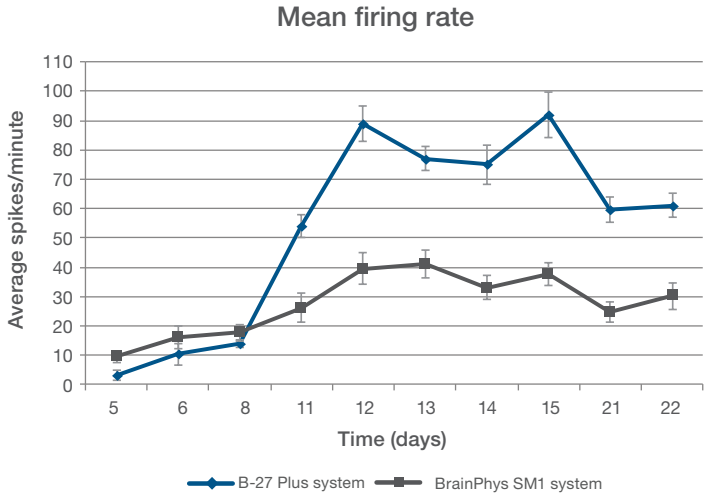

Figure 7. The B-27 Plus system promotes higher levels of functional activity in factor-driven induced neurons. Multi-electrode array (MEA) data performed by scientists at Fulcrum Therapeutics comparing functional activity of hiPSC-induced neurons matured in the B-27 Plus system to the BrainPhys SM1 culture system.

the B-27 Plus or BrainPhys SM1 system. Overall neuronal activity, measured by mean firing rate, was significantly higher in the B-27 Plus system compared to the BrainPhys SM1 system.

Additional guidance

We have provided guidance and demonstrated the benefits of using the B-27 Plus Neuronal Culture System with PSC-derived neurons. For more detailed guidance, please see the B-27 Plus PSC-derived neuron user guide.

Find out more at **thermofisher.com/b27plus**

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