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Making the switch to B-27 Plus Neuronal Culture System

Protocol guidance for success

For more than 25 years scientist have relied on the "classic" Gibco™ B-27™ Supplement to enable their research by improving the *in vitro* neural cell culture environment. Throughout the years, researchers modified their protocols and workflows to optimize performance of their cell culture system. Now, you can streamline your neural culture workflow with the Gibco™ B-27™ Plus Neuronal Culture System. This system includes a simplified protocol, helps improve reliability and consistency of your experiments, and increases neuronal cell survival by more than 50% over classic B-27 Supplement.

This document is intended to provide guidance to ensure successful trial of the B-27 Plus Neuronal Culture System for primary neuron culture. This next-generation culture system offers these key strengths:

- Improves neuronal health and supports longer cell viability at lower seeding densities versus classic B-27 Supplement (Guidance #1)
- Helps eliminate or reduce the need to add additional components, including serum (Guidance #2)

Guidance #1. Fewer cells required: improved neuronal health at lower seeding densities

Gibco™ B-27 Plus Supplement, when combined with Gibco™ Neurobasal™ Plus Medium, offers up to 50% higher survival rate of primary neurons compared to classic B-27 Supplement and Gibco™ Neurobasal™ Medium. Therefore, when you are plating neurons in B-27 Plus system, you will need fewer cells per well in your experiments than you did when classic B-27 Supplement and Neurobasal Medium was used. See our recommendations below for seeding neurons when switching from B-27 Supplement to B-27 Plus system (Table 1). With fewer cells, the B-27 Plus system supports greater viability and quality of neurons with fewer cells required (Figure 1).

Table 1. Recommended seeding densities for primary neurons.

		Seeding density		
Source	**Medium**	**Low (cells/cm²)**	**Medium (cells/cm²)**	**MEA application High (cells/drop)**
Rat	B-27 classic*	≥ 40,000	≥ 90,000	160,000
	B-27 Plus system	20,000	60,000	80,000
Mouse	B-27 classic*	≥ 60,000	≥ 100,000	120,000
	B-27 Plus system	30,000	60,000	60,000
Feeding schedule: (Suggested half-volume change per feed)		1–2 times weekly	Every 3–4 days	Every 2–3 days

* B-27 classic = B-27 Supplement with Neurobasal Medium

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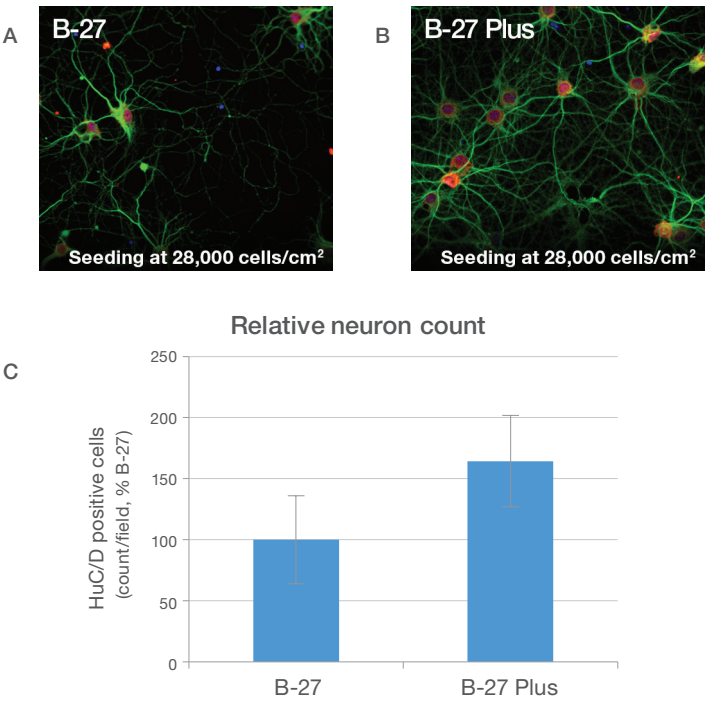

Figure 1. Greater viability and quality of primary neurons with fewer cells: mouse cortical neurons on culture day 14. Cell were plated at 28,000 cells/cm² and maintained in either **A)** "classic" B-27 Supplement and Neurobasal Medium or **B)** the B-27 Plus Neuronal Culture System (B-27 Plus Supplement with Neurobasal Plus Medium). The number of neurons **(C)** was determined by automated image capture and analysis on the Thermo Scientific™ CellInsight™ CX5 HCS Platform with the Thermo Scientific™ Studio™ Cell Analysis Software. Neurons were stained with MAP2 (green) and HuC/D (red) antibodies. Nuclei were labeled with Invitrogen™ Hoechst 33342 (blue).

Guidance #2. Eliminate the need for additional components

Serum is not required when using the B-27 Plus Neuronal Culture System. Removing serum from your primary neuron culture benefits you by reducing cost and protocol complexity for more reliable experimental results. To observe improved survival, neurite outgrowth, and neural network activity with the B-27 Plus system, we strongly recommend avoiding the use of serum. Scientists at Axion Biosystems performed multi-electrode array (MEA) analysis (Figure 2) on (cryopreserved) primary rat cortical neurons and demonstrated consistently superior and high-quality electrophysiological recordings in neurons maintained in B-27 Plus system versus classic B-27 Supplement and Neurobasal Medium with serum.

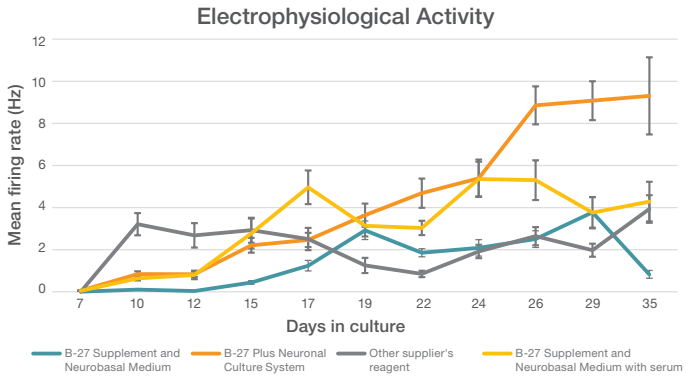

Figure 2. The B-27 Plus system promotes better electrophysiological activity versus classic B-27 Supplement with serum. The B-27 Plus system (orange) promoted a higher degree of firing activity as the network matured, as quantified via mean firing rate. Excitability of a neural network was quantified by the mean firing rate, defined as the total number of action potentials detected per second.

Seeing the difference in your culture

The benefits of the B-27 Plus system are typically observed within 1–3 weeks, including increased neuronal survival, accelerated neurite outgrowth, and improved neural network activity and maturation in primary neurons. Follow the above protocol guidance and B-27 Plus Neuronal Culture System protocol (**thermofisher.com/b27plus**) to observe these benefits in your primary neuron cultures.

When should you see benefits?	
Key benefit	**Typical time observed**
Increased neuronal survival	≥ 2 weeks
Neurite outgrowth	Within 1 week
Functional network activity (MEA)	2–3+ weeks

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

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