# B-27 Plus Neuronal Culture System enables superior primary neuron cultures

## For improved survival, maturation, and neural network activity

For over 25 years, the classic Gibco™ B-27™ Supplement and Gibco™ Neurobasal™ Medium have set the standard in neuronal cell culture reagents. The Gibco™ B-27™ Plus Neuronal Culture System, composed of the Gibco™ B-27™ Plus Supplement (Cat. No. A3582801) and Gibco™ Neurobasal™ Plus Medium (Cat. No. A3582901), improves upon the classic culture environment through raw material and manufacturing upgrades and minor formulation modifications. Together, these small changes yield big results.

### Improved neuronal health

#### Better long-term culture

Maintaining healthy long-term cultures (3 weeks and beyond) of primary rodent neurons can be challenging, as these cells are quite sensitive and tend to undergo progressive cell death over time. Figure 1 highlights the benefits of the B-27 Plus system with mouse cortical neurons. Note that cell numbers and the extent of MAP2 staining are both significantly improved using the B-27 Plus system.

#### Improved neurite outgrowth

One early indicator of the quality of neuronal cultures that can be seen soon after plating is neurite outgrowth. This process of neurites extending from a neuron (development of axons and dendrites) occurs during neuronal maturation. Figure 2 illustrates the improved neurite outgrowth seen before a week in culture using the B-27 Plus system.

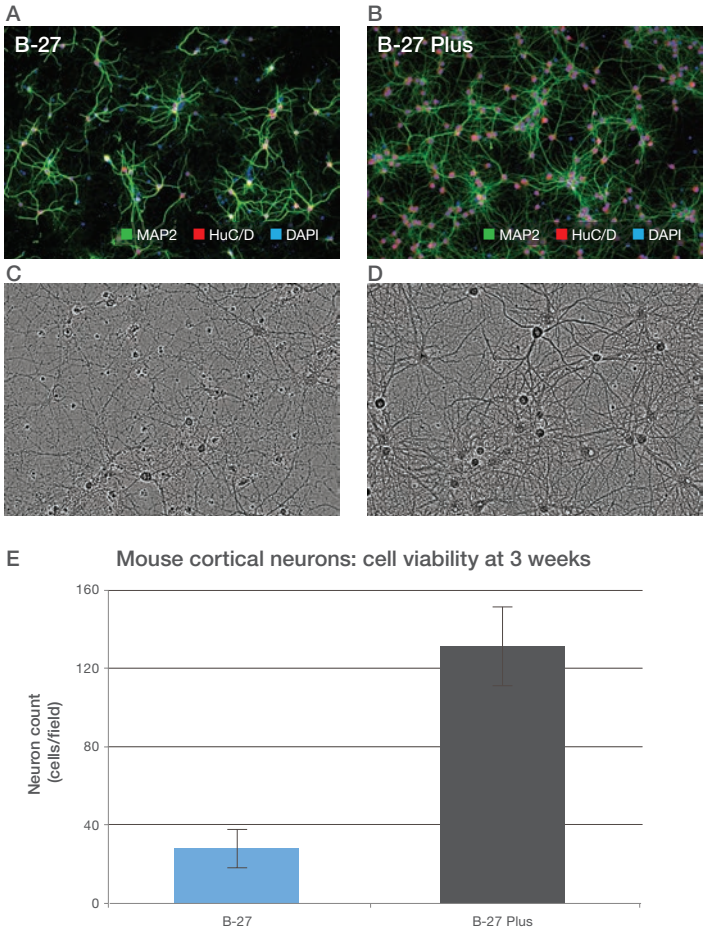


**Figure 1. Improved survival and maturation of mouse cortical neurons with the B-27 Plus system.** Cryopreserved cells were plated at 60,000 cells/cm$^2$ and cultured in either the classic B-27 Supplement and Neurobasal Medium **(A** and **C)** or the B-27 Plus Neuronal Culture System **(**B-27 Plus Supplement and Neurobasal Plus Medium; **B** and **D**). The number of neurons **(E,** n = 4**)** was determined by automated image capture and analysis on the Thermo Scientific™ CellInsight™ CX5 High Content Screening (HCS) Platform. Neurons were stained for MAP2 (green) and HuC/D (red), and nuclei were labeled with DAPI (blue).

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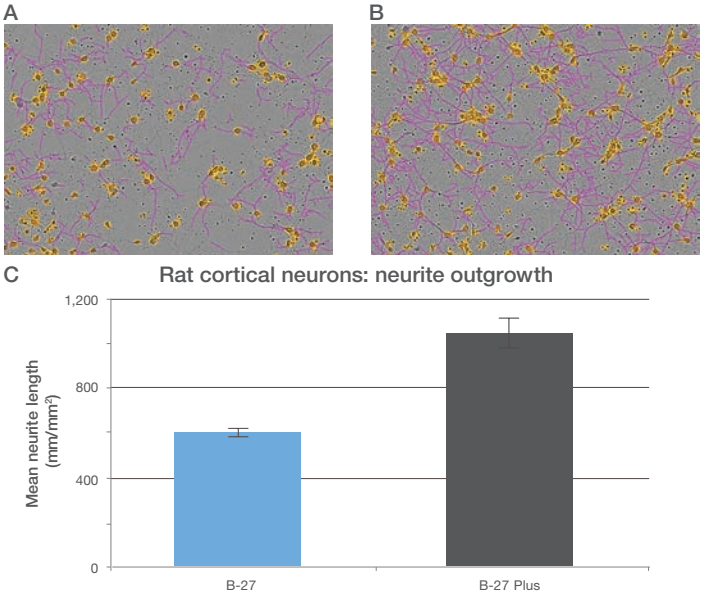


**Figure 2. Morphometric analysis of neurite outgrowth in rat cortical neurons.** Cryopreserved cells were plated at 5 x $10^4$ cells/$cm^2$ and cultured in either **(A)** classic B-27 Supplement and Neurobasal Medium or **(B)** the B-27 Plus Neuronal Culture System (B-27 Plus Supplement and Neurobasal Plus Medium). Six days following plating, neurite lengths were calculated using an Essen BioScience IncuCyte™ Zoom System for live-cell imaging. **(C)** Quantification of neurite length (indicated by the purple lines in the microscopy images) is shown relative to cell bodies (yellow).

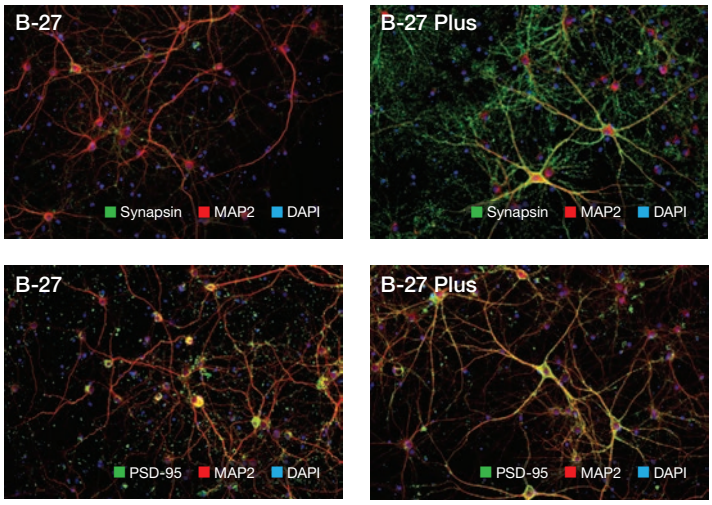


**Figure 3. Enhanced expression of pre- and postsynaptic markers in rat cortical neurons (RCNs) matured in the B-27 Plus system.** RCNs were cultured as indicated for 28 days and then immunostained for MAP2 (red), synapsin (green), and PSD-95 (green). Nuclei were labeled with DAPI (blue).

## Increased synaptic complexity

Many changes occur during neuronal maturation, two hallmarks of which are axonal outgrowth and synapse formation, which increase neurite network density and complexity. One common presynaptic marker is the synapsin family of proteins involved in the release of neurotransmitters at synapses. Postsynaptic structures are commonly visualized using postsynaptic density protein 95 (PSD-95), a membrane-associated guanylate kinase that plays a number of roles in modulating postsynaptic function, including direct interactions with neurotransmitter receptors and potassium channels. The presence of synapsin and PSD-95 indicates functionally mature synaptic connections. We show here that Gibco™ Primary Rat Cortex Neurons (RCNs) (Cat. No. A10840) cultured in the B-27 Plus system exhibit significantly more extensive expression of both synapsin and PSD-95 than cells in classic B-27 and Neurobasal medium (Figure 3).

## Impact on neuronal subtypes

Preparations of neurons from rodent brains contain different types of cells, including excitatory (glutamatergic) and inhibitory (GABAergic) neurons, and a subset of GABAergic interneurons that are positive for parvalbumin (PV). *In vivo*, the numbers of excitatory and inhibitory neurons are tightly regulated, and their interactions are the foundation for neural network regulation. Further, PV interneurons play a critical role in controlling synchronous activity across networks. *In vitro*, neuronal survival and robust maturation are vital to neuronal subpopulation maintenance and the formation of networked and synchronous activity in primary cortical and hippocampal cultures.

The impact of culture conditions on subpopulations of excitatory and inhibitory neurons in RCNs is shown in Figure 4, using comparisons between 28-day cultures in the classic B-27 and Neurobasal medium and the B-27 Plus system. The total numbers of neurons were determined by HuC/D-positive cells. These cells were further quantified into neuronal subpopulations of glutamatergic neurons (VGLUT2-positive), GABAergic neurons (GABA-positive), and PV-positive interneurons. Quantitative image analysis showed approximately 50% more HuC/D-positive cells in the B-27 Plus system. 90% of all neurons in the classic B-27 system stained positive for VGLUT2, compared to 81% in the B-27 Plus System. Cultures in the classic B-27 system had 12% GABA-positive neurons, versus 14% in the B-27 Plus system. Strikingly, the percentage of PV interneurons was less than

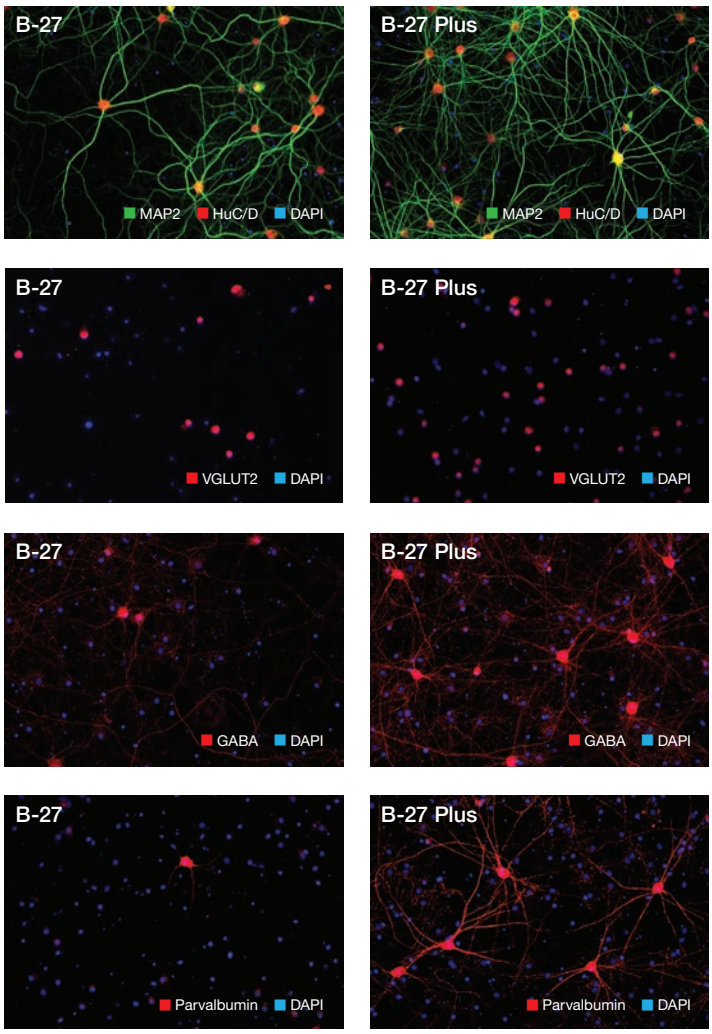


**Figure 4. Defining neural subtypes in cultures of primary rat neurons.** RCNs were cultured for 28 days in either the classic B-27 system (left column) or the B-27 Plus system (right column) and then immunostained for MAP2 (green) and HuC/D (red), VGLUT2 (red), GABA (red), or parvalbumin (red). Nuclei were labeled with DAPI (blue).

1% in the classic B-27 system compared to 7% in the B-27 Plus system. These results are in line with estimates of 80% glutamatergic and 20% GABAergic identity for cortical neurons. Qualitative analysis of the MAP2, GABA, and PV staining indicate significantly more developed neurite staining in the B-27 Plus system, suggesting enhanced maturation of the neurons and their subpopulations. Taken together, these data indicate significant improvements in the maintenance and maturity of neuronal subpopulations in the B-27 Plus system.

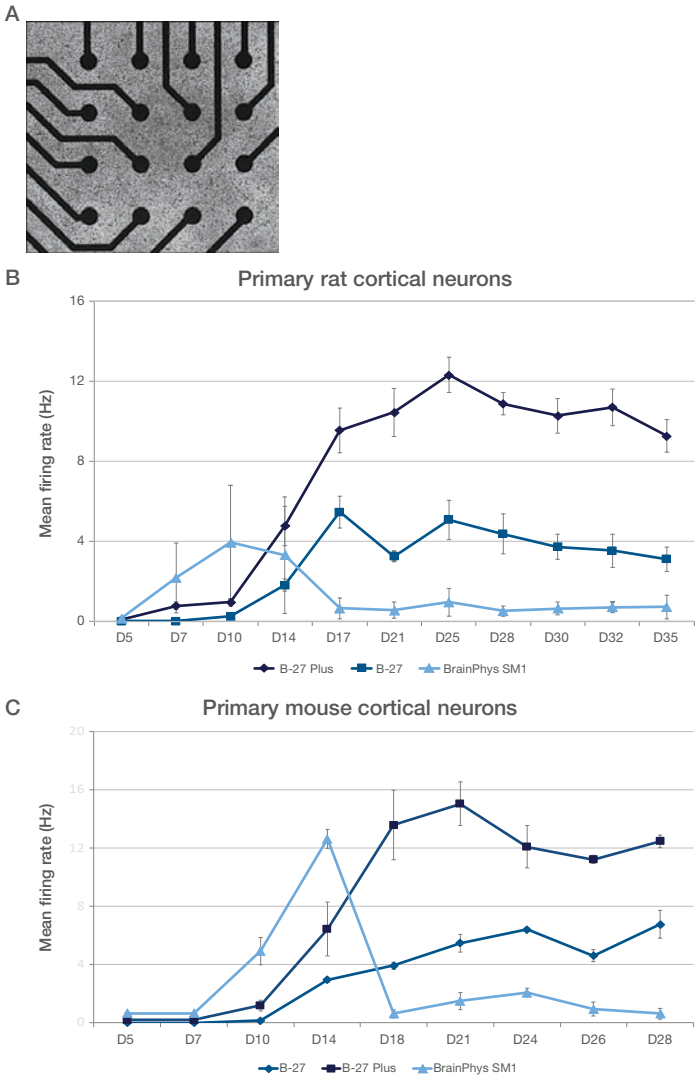


**Figure 5. Improved electrophysiology in primary rodent neurons with the B-27 Plus system. (A)** Photomicrograph of cultured primary mouse neurons at day 22 on a MEA grid. Rat **(B)** or mouse **(C)** cortical neurons were seeded as high-density drops on 24-well MEA plates and maintained in classic B-27, B-27 Plus, and BrainPhys™ Neuronal Medium and SM1 Kit culture media systems for up to 35 days. Spontaneous electrical activity, recorded as mean firing rate, was measured at regular intervals using the Axion BioSystems Maestro Pro™ MEA platform.

## Improvement of electrophysiological activity

Another measure of neural maturation is the expression and activity of voltage-gated ion channels, which underlie the generation of action potentials of individual neurons and broader networking activity across groups of neurons. Multielectrode array (MEA) systems, which utilize specialized cultureware, enable noninvasive, direct measurement of the electrical activity of a group of cells by culturing neurons in close proximity to sensing electrodes (Figure 5A). Using this approach, the spontaneous activity

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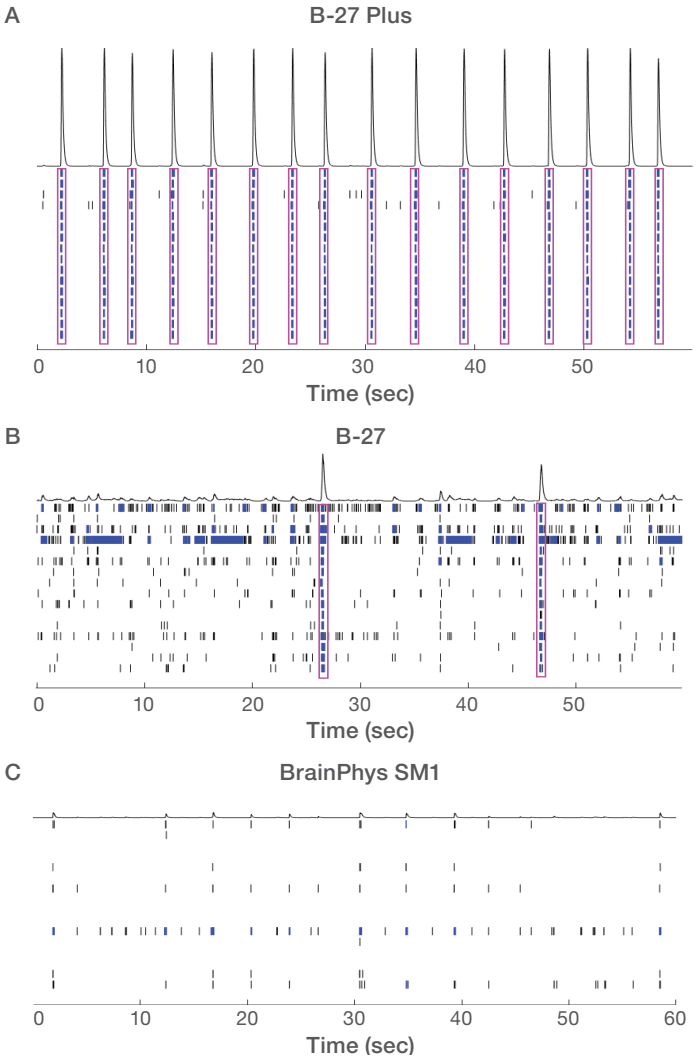


**Figure 6. Analysis of network activity by MEA.** Raster plots demonstrating synchronized bursting in day 28 RCN cultures in **(A)** the B-27 Plus system, **(B)** the classic B-27 system, or **(C)** a leading supplier's system. The spiked traces indicated calculated bursts of activity, which are defined by simultaneous electrical activity measured at each sensing electrode (16 total for the B-27 Plus system, versus two in the classic B-27 system and none in the system of the other leading supplier; highlighted in purple).

(measured by mean firing rate) of rat and mouse cortical neurons was measured over time, with cells cultured in the B-27 Plus System showing much higher and sustained activity than those in the classic B-27 system or a leading supplier's medium (Figure 5B, C).

Additional types of data can be extracted from the MEA recordings, including calculations of network activity and synchrony of bursting, in which data from each individual electrode in a well is looked at simultaneously. This enables estimation of network connectivity as well insight into the quality of the network itself. Figure 6 shows representative bursting data from a one-minute recording of day 28 RCNs cultured as indicated.

The bursting data with RCN culture in the B-27 Plus system show remarkable consistency and synchrony, with very little inter-burst noise. Bursts are driven by the coordinated activities of both excitatory and inhibitory neurons. The B-27 Plus system uniquely enables the generation of a stable "window" of network synchrony, allowing investigators to interrogate this complex neural interplay.

## Conclusion

The classic B-27 system has been a mainstay of neurobiology research for almost three decades. The B-27 Plus system builds upon the original supplement and basal medium, and provides significant improvements in supporting primary neuron health, maturation, and function. This new formulation enables researchers to improve the quality of their neural cultures and thus the types of data obtained from them. Examples highlighted here include overall maintenance of high-quality cultures of primary rodent neurons, enabling a stable window for interrogating actions underlying activity for up to 4 weeks. The basis for this improved activity lies at least in part in the denser neurite networks and higher synaptic density enabled in the B-27 Plus system. Collectively, these data highlight the key benefits of the B-27 Plus and Neurobasal Plus culture system.

### Ordering information

| Product | Cat. No. |
|---|---|
| B-27 Plus Neuronal Culture System | A3653401 |

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

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