Maximize survival of neurons derived from primary and stem cells

Introducing the Gibco B-27 Plus Neuronal Culture System.

Functional *in vitro* studies of neurons and neural networks require that the cells be maintained for prolonged periods of time at an optimal density. For primary rodent neurons, full functionality and maturation require maintenance *in vitro* for approximately 21 days, whereas human induced pluripotent stem cell (iPSC)–derived neurons require several weeks to months in culture. For more than 25 years, scientists have relied on Gibco™ B-27™ Supplement and Gibco™ Neurobasal™ Medium for a variety of neuronal culture applications [1].

Maintain neuronal cultures longer

Scientists are now seeking to maintain a higher density of neurons over longer periods of time than B-27 Supplement and Neurobasal Medium were originally designed to support. Part of the reason for these more stringent growth conditions is the advent of human iPSC-derived neurons, which grow and mature more slowly than primary rodent neurons. To address this need, we have developed the Gibco™ B-27™ Plus Neuronal Culture System—a serum-free neuronal culture system consisting of B-27 Plus Supplement and Neurobasal Plus Medium—that reliably enables the highest survival rates of primary and stem cell–derived neurons.

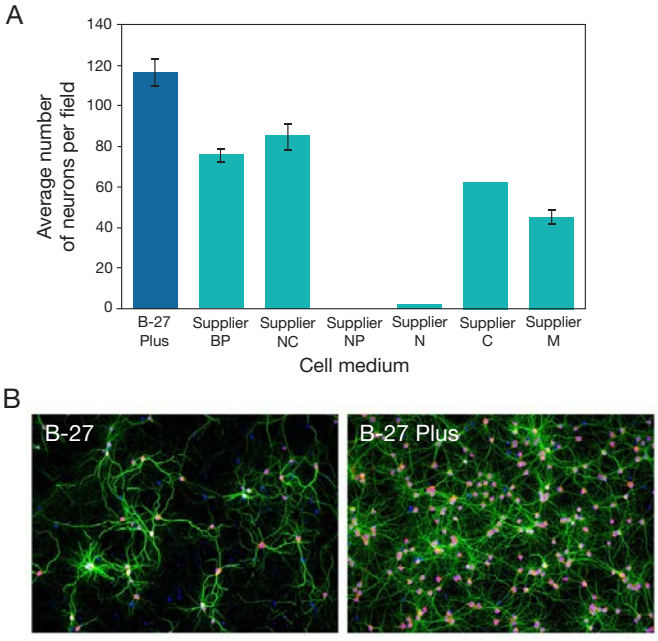

Figure 1. The B-27 Plus Neuronal Culture System promotes the highest survival rates of primary rodent neurons in culture. (A) Cryopreserved mouse cortical neurons (Cat. No. A15586) were maintained for 21 days in the indicated media. Neuronal survival was measured by immunofluorescence labeling of the neuronal somatic marker HuC/D using an anti-HuC/HuD monoclonal antibody (clone 16A11, Cat. No. A21271) in conjunction with Invitrogen™ Alexa Fluor™ 594 anti–mouse IgG secondary antibody (Cat. No. A11005). The bar graph shows the average results of three experiments in which the Gibco™ B-27™ Plus Neuronal Culture System (Cat. No. A3653401) was compared with other commercially available products. **(B)** Mouse cortical neurons, cultured for 21 days with either the B-27 Plus Neuronal Culture System or the classic B-27 Supplement and Neurobasal Medium, were immunostained for MAP2 (green) and HuC/HuD (red); nuclei were counterstained with DAPI nucleic acid stain (blue). These fluorescence images are representative fields that illustrate the improved neuronal survival using the B-27 Plus Neuronal Culture System.

Optimized formulations for neuronal culture

Leveraging our experience as the sole provider of B-27 supplements, we have optimized the formulation of B-27 Plus Supplement and Neurobasal Plus Medium, implemented more stringent requirements for raw materials, and improved our manufacturing, validation, and quality control processes. The optimized products work synergistically as a system, significantly improving upon the classic combination of B-27 Supplement and Neurobasal Medium and also outperforming other commercially available media in terms of neuronal survival and long-term maintenance (Figure 1).

In addition, the B-27 Plus Neuronal Culture System significantly accelerates neurite outgrowth in short-term neuronal cultures (Figures 2A and 2B) and improves the electrical activity in long-term cultures relative to the classic B-27 Supplement and Neurobasal Medium (Figure 2C). The accelerated neurite outgrowth and increase in spontaneous firing, together with a concomitant increase in synapsin staining (Figure 2D) for neurons grown using the B-27 Plus system, indicate the formation of functional synapses.

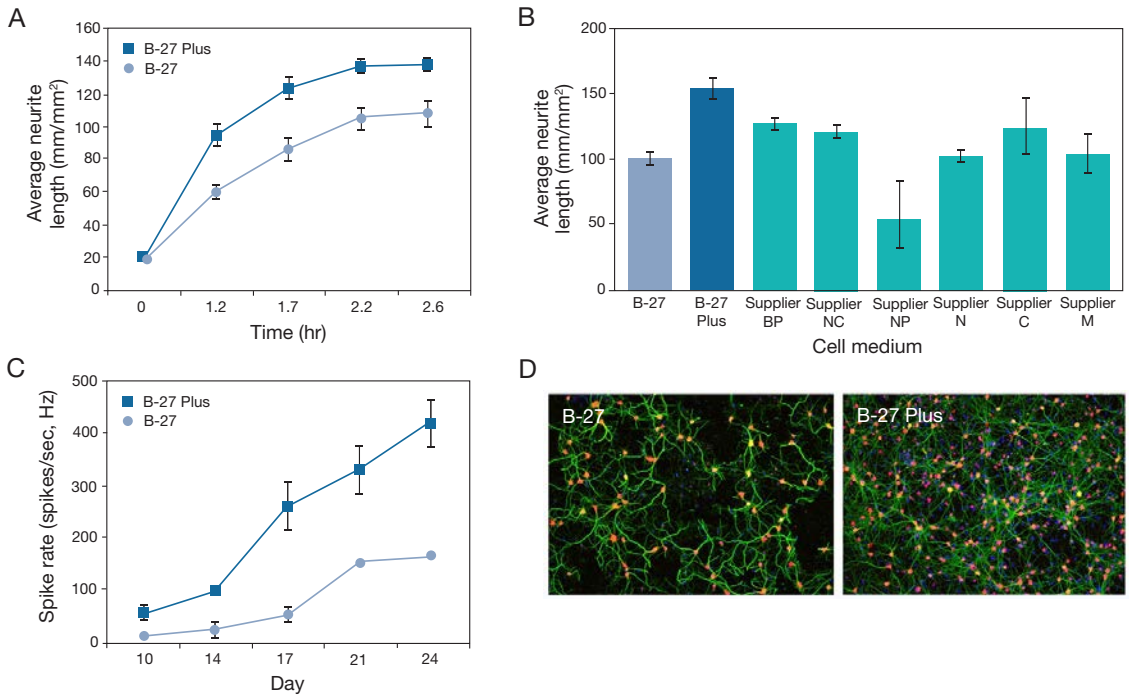

Figure 2. As compared with B-27 Supplement and Neurobasal Medium, the B-27 Plus Neuronal Culture System produces significantly improved maturation and electrical activity in primary rodent neurons. (A, B) Cryopreserved mouse cortical neurons (Cat. No. A15586) were maintained in the indicated media systems, and neurite outgrowth was quantified using differential interference contrast images. **(A)** The Gibco™ B-27™ Plus Neuronal Culture System showed accelerated neurite outgrowth when compared with that obtained using the original combination of B-27 Supplement and Neurobasal Medium. **(B)** When compared with other commercially available media systems, the B-27 Plus Neuronal Culture System also produced the longest neurite lengths after 7 days. **(C, D)** Cryopreserved rat cortical neurons (Cat. No. A1084002) were maintained for over 3 weeks in either the classic B-27 Supplement and Neurobasal Medium or the B-27 Plus Neuronal Culture System. **(C)** Cells were grown in 12-well MEA plates, and electrical activity recordings were initiated on *in vitro* day 10 and continued to day 24. The graph of spike rate vs. time shows the increase in spontaneous electrical activity from day 10 to day 24; each data point is an average of three wells. **(D)** Rat cortical neurons, cultured for 28 days with either the B-27 Plus Neuronal Culture System or the classic B-27 Supplement and Neurobasal Medium, were immunostained for MAP2 (green) and synapsin (red); nuclei were counterstained with DAPI nucleic acid stain (blue). These fluorescence images are representative fields that illustrate the increased synapse formation using the B-27 Plus Neuronal Culture System.

Learn more about B-27 Plus neuronal culture

The B-27 Plus Neuronal Culture System promotes high survival rates of primary and stem cell–derived neurons in both short- and long-term culture, which enables successful downstream applications utilizing these cells (see front cover image). In protocols used to differentiate, maintain, and mature neurons, the optimized B-27 Plus Supplement and Neurobasal Plus Medium should be used in place of the original versions of these products. The B-27 Plus Neuronal Culture System should not be used for neural stem cell expansion or in place of other specialized versions of B-27 supplements and Neurobasal media (e.g., B-27 supplements minus vitamin A or minus insulin, or Neurobasal-A media). B-27 Plus Supplement and Neurobasal Plus Medium are offered in the same volumes and concentrations as the classic products, which will continue to be available. Learn more at **thermofisher.com/b27bp76**. ■

Reference

1. Brewer GJ, Torricelli JR, Evege EK et al. (1993) *J Neurosci Res* 35:567–576.

Product	Quantity	Cat. No.
B-27 Plus neuronal culture media		
B-27™ Plus Neuronal Culture System	1 kit	A3653401
B-27™ Plus Supplement (50X)	10 mL	A3582801
Neurobasal™ Plus Medium	500 mL	A3582901
Classic B-27 neuronal culture media		
Gibco™ Neural Cell Culture Starter Kit	1 kit	A32116
B-27™ Supplement (50X), minus antioxidants	10 mL	10889038
B-27™ Supplement (50X), minus insulin	10 mL	A1895601
B-27™ Supplement (50X), minus vitamin A	10 mL	12587010
B-27™ Supplement (50X), serum free	10 mL	17504044
CTS™ B-27™ Supplement, XenoFree	10 mL	A1486701
CultureOne™ Supplement (100X)	5 mL	A3320201