Tools for 2D and 3D neuronal cell culture
From the B-27 Plus Neuronal Culture System to fluorescent probes for cell analysis.

Traditional culture and analytical methods in the neuroscience field have long relied on rodent cell model systems to emulate the behaviors of higher mammalian neural network activity, with the aim of discovering, characterizing, and testing pharmaceuticals for safety and toxicology. These and most excitable, postmitotic cell types are typically dissociated from freshly prepared tissues or cryopreserved for later study using a variety of media and extracellular matrix formulations intended to recapitulate native or in vivo conditions.

To this end, the commercial availability of large-scale batches of quality-controlled, cryopreserved primary cell types has helped advance the study of brain and nerve function. Also available are improved media formulations specifically developed to increase neuronal survival and enhance maturation of neuronal phenotypes in extended cultures of primary and induced pluripotent stem cell (iPSC)-derived neurons, as well as functional probes designed for use with 2D and 3D neuronal cultures.

Figure 1 (above). Visualizing a mouse cortical neuron culture. Cryopreserved Gibco™ Primary Mouse Cortical Neurons (Cat. No. A15586) were grown in culture for 3 weeks using the Gibco™ B-27™ Plus Neuronal Culture System (Cat. No. A3653401). Cells were fixed and labeled with Invitrogen™ NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606), anti-β-tubulin mouse monoclonal antibody (Cat. No. R32-2600) in conjunction with Invitrogen™ Alexa Fluor™ 488 goat anti–mouse IgG antibody (Cat. No. A11029), and Invitrogen™ ActinRed™ 555 ReadyProbes™ Reagent (Cat. No. R37712). Cells were mounted in Invitrogen™ ProLong™ Glass Antifade Mountant (Cat. No. P36960) and imaged on an Invitrogen™ EVOS™ FL Auto 2 Imaging System using a 60x oil immersion objective.
Advancing neuronal culture

For over 25 years, researchers have relied on Gibco™ B-27™ Supplement and Gibco™ Neurobasal™ Medium for their neuronal culture applications [1]. The original serum-free B-27 Supplement was developed for the long-term culture of rat hippocampal and cortical neurons. However, the publication history of B-27 supplements demonstrates that researchers are expanding their use far beyond these applications. Progress in the development of iPSC-derived neurons has led to the need to maintain a higher density of neurons over longer periods of time than B-27 Supplement and Neurobasal Medium were designed to support. We have applied our decades of experience with culture media to develop the Gibco™ B-27™ Plus Neuronal Culture System—a serum-free culture system that provides increased neuronal survival, accelerated neurite outgrowth, and improved electrophysiological activity and maturation of neurons.

A closer look at neuronal subtypes

The B-27 Plus Neuronal Culture System improves upon the classic culture environment through both raw material and manufacturing upgrades and minor formulation modifications. Here we look at how improved neuronal culture conditions affect subpopulations of neurons in primary neuronal cultures and the functional implications of enhanced subpopulation survival and maturity. 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 rat cortical neurons is shown in Figure 2, using comparisons between 28-day cultures grown in the classic B-27 Supplement and Neurobasal Medium and in the B-27 Plus system. The total numbers of neurons in each culture were determined by counting HuC/D-positive cells. Using neuronal subpopulation–specific antibodies, we further characterized these cells as glutamatergic neurons (VGLUT2-positive), GABAergic neurons (GABA-positive), and PV-positive interneurons.

Quantitative image analysis performed on the Thermo Scientific™ CellInsight™ CX5 High-Content Screening (HCS) Platform showed approximately 50% more HuC/D-positive cells in the B-27 Plus system than in the classic B-27 system. In addition, 90% of all neurons in the classic B-27 system stained positive for VGLUT2, compared with 81% in the B-27 Plus system, and 12% of the neurons in the B-27 cultures were GABA-positive neurons versus 14% in the B-27 Plus system. Strikingly, the percentage of PV-positive interneurons was less than 1% in the classic B-27 system, compared with 7% in the B-27 Plus system. These results are in line with estimates of 80% glutamatergic and 20% GABAergic for cortical neurons in vivo. Qualitative analysis of the MAP2, GABA, and PV staining indicates significantly more staining of developed neurites in cells cultured 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 maturation of neuronal subpopulations in the B-27 Plus system.

Challenges to neuronal maturation and analysis of 2D and 3D cultures

One of the principal barriers to generating useful data with neural culture systems is the length of time required for stem cell–derived and primary cultures to reach maturation for downstream functional analysis. Typically, culture medium must be refreshed 2–3 times
Neuronal spheroids were cultured using Gibco™ Neurobasal™ (Cat. No. 21103049) or Gibco™ Neurobasal™ Plus (Cat. No. A28920) Medium, with and without Gibco™ CultureOne™ Supplement (Cat. No. A3323201), in Thermo Scientific™ Nunc™ Sphera™ 96U-Well Microplates, and then stained with Invitrogen™ Tubulin Tracker™ Deep Red reagent (Cat. No. T34077). Images were generated using a 10x objective on the Thermo Scientific™ CellInsight™ CX7 High-Content Analysis (HCA) Platform. Neuronal spheroids were labeled with Tubulin Tracker Deep Red and Invitrogen™ NucBlue™ Live ReadyProbes™ (Cat. No. R37605) reagents. Cells were imaged in Gibco™ HBSS containing calcium and magnesium (Cat. No. 14025134), supplemented with probenecid (Cat. No. P36400). Images were generated using an Invitrogen™ EVOS™ FL Auto 2 Imaging System with a 20x objective and an Invitrogen™ EVOS™ Cy5 Light Cube.

Explore 2D and 3D culture and analysis
Thermo Fisher Scientific offers a suite of culture media, cultureware, cell analysis reagents, and fluorescence instrumentation for 2D and 3D neuronal cell cultures. Learn more at thermofisher.com/neurobasal.

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