Neurobiology

Building more relevant and reliable neuronal models with Neurobasal PhysioPlus Medium and B-27 Plus Supplement

Introduction

In vitro neuronal models have become indispensable tools for studying nervous system development, disease mechanisms, target biology, and therapeutic interventions. Researchers increasingly rely on primary neurons and human induced pluripotent stem cell (hiPSC)-derived neurons to create physiologically relevant models that can support applications ranging from routine culture maintenance to advanced functional assays.

The predictive value of these models depends on the ability of cultured neurons to survive, mature, establish functional connectivity, and generate stable electrophysiological activity. While advances in stem cell technologies have expanded access to human-relevant neuronal systems, culture media formulations remain a critical determinant of experimental success. Suboptimal culture conditions can limit neuronal maturation, compromise long-term viability, and introduce variability into functional assays.

To address these challenges, we developed Gibco™ Neurobasal™ PhysioPlus Medium, an advanced culture medium designed to better reflect the extracellular environment of the central nervous system (CNS). Neurobasal PhysioPlus Medium features physiological osmolarity, optimized glucose concentration, and refined levels of neuroactive components, including amino acids. When combined with Gibco™ B-27™ Plus Supplement, the resulting neuronal culture system supports robust neuronal health while promoting earlier and more sustained functional maturation.

This application note demonstrates how the Neurobasal PhysioPlus Medium and B-27 Plus Supplement system supports diverse neuronal models and helps to enable reliable functional neuroscience workflows.

Neurobasal PhysioPlus Medium supports neuronal health across cell types

A neuronal culture system must first provide a strong foundation for neuronal survival and maintenance. To evaluate the performance of Neurobasal PhysioPlus Medium, multiple neuronal model systems were examined, including hiPSC-derived neurons generated through distinct differentiation approaches, as well as primary rodent cortical neurons.

Across neuronal models, cultures maintained in Neurobasal PhysioPlus Medium with B-27 Plus Supplement exhibited excellent morphology and long-term viability. Neurons developed extensive neurite networks while maintaining healthy cell bodies throughout extended culture periods.

Human iPSC-derived neurons

hiPSC-derived glutamatergic neurons cultured in Neurobasal PhysioPlus Medium demonstrated improved survival compared with a leading system from another supplier (Figure 1). By day 28, cultures maintained in Neurobasal PhysioPlus Medium and B-27 Plus Supplement exhibited 32% higher survival while preserving healthy neuronal morphology. High survival and healthy morphology were observed for iPSC-derived neurons from a variety of sources.

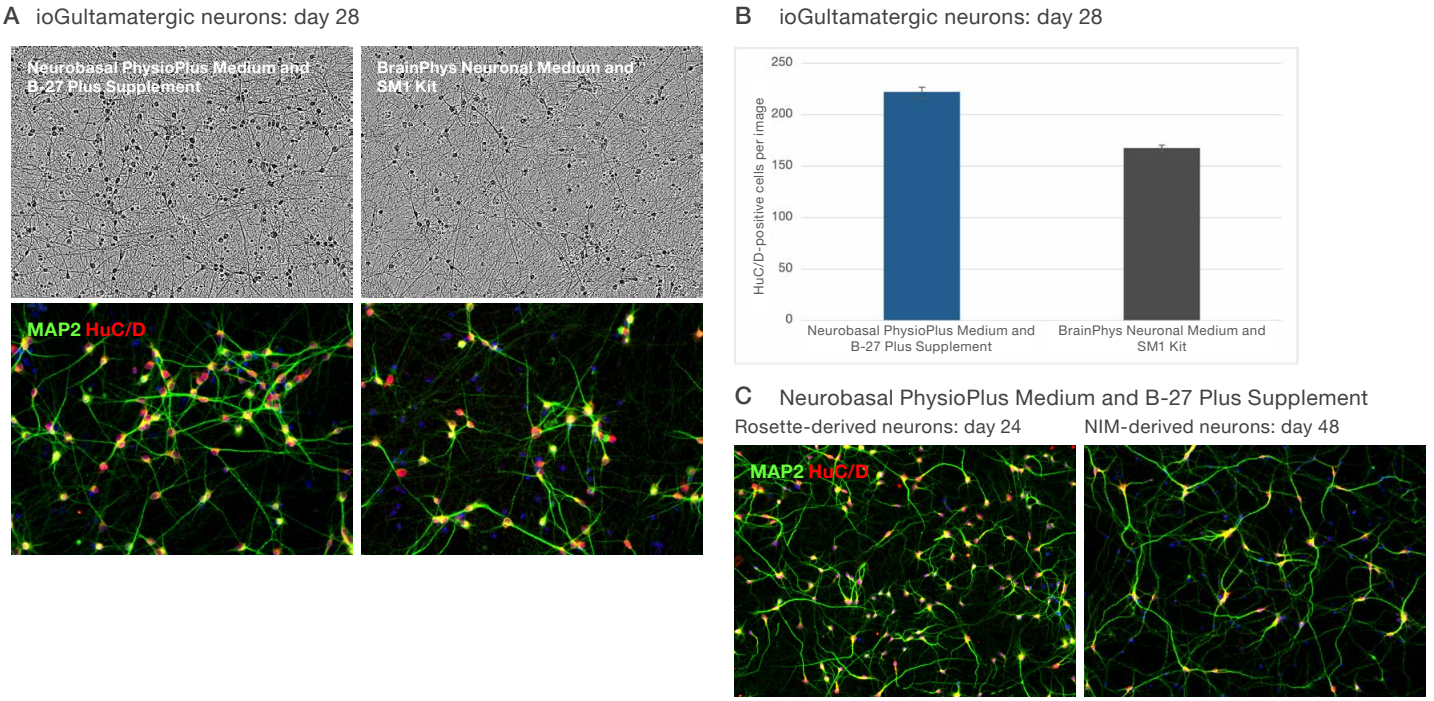

Figure 1. Long-term survival of PSC-derived neurons cultured in Neurobasal PhysioPlus Medium. hiPSC-derived glutamatergic neurons from bit.bio (ioGlutamatergic neurons) were seeded on poly-d-lysine and laminin double-coated plates in the recommended neuronal induction medium for 5 days of neuronal induction. After neuronal induction, medium was changed to Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement or the BrainPhys™ Neuronal Medium and SM1 Kit (STEMCELL Technologies). To assess the impact of neuronal culture systems on neuronal survival, the recommended growth factors for maturation were not included in the medium after day 5. **(A)** After 28 days, phase images demonstrate healthy neuronal cultures in Neurobasal PhysioPlus Medium with intact cell bodies and significant neurite outgrowth while the condition with the BrainPhys and SM1 system shows degrading cell bodies. **(B)** At day 28, cells were fixed and stained with antibodies for MAP2 and HuC/D. Neuronal survival was assessed by quantifying the number of HuC/D-positive cells. Neurobasal PhysioPlus Medium with B-27 Plus Supplement showed >32% increase in neuronal survival compared to the BrainPhys and SM1 system. **(C)** Rosette-derived neurons and neural induction medium (NIM)-derived neurons (directed differentiation) were cultured for 24 and 48 days, respectively, in Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement. Fluorescent staining suggests Neurobasal PhysioPlus Medium supports long-term maintenance of PSC-derived neurons generated from different derivation methods.

Primary rat cortical neurons

Primary cortical neurons cultured in Neurobasal PhysioPlus Medium and B-27 Plus Supplement maintained robust neuronal networks through at least 4 weeks in culture (Figure 2). In contrast, a culture system from another supplier exhibited substantial deterioration during long-term maintenance.

At day 21, Neurobasal PhysioPlus Medium supported approximately 69% greater neuronal survival compared with the alternative system.

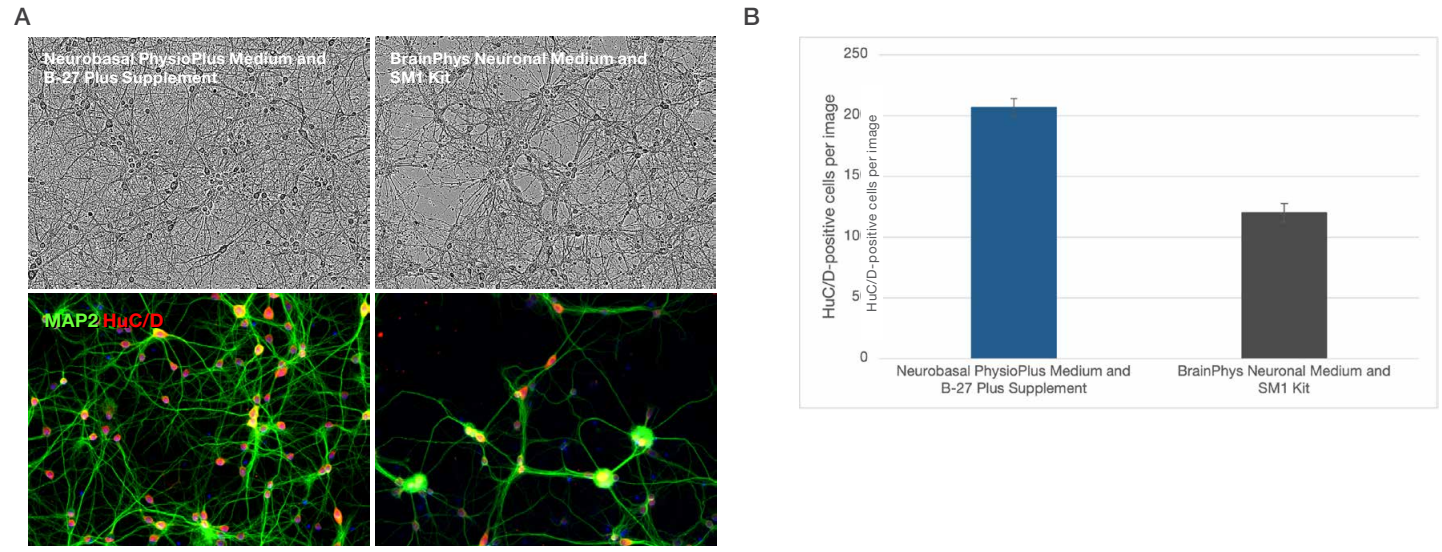

Figure 2. Survival of primary rat cortical neurons in different culture systems. Rat cortical neurons (E18) were plated and maintained in Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement or the BrainPhys Neuronal Medium and SM1 Kit for 28 days. **(A)** Phase images show robust neuronal cultures at day 28 for cells cultured in Neurobasal PhysioPlus Medium. The BrainPhys and SM1 system shows significant neuronal detachment. Cells were fixed and stained with MAP2 and HuC/D antibodies. **(B)** Neuronal survival was assessed by quantifying the number of HuC/D-positive cells.

Morphological maturation of neurons

Beyond survival, neurons cultured in Neurobasal PhysioPlus Medium developed hallmark characteristics of mature neuronal networks. Immunocytochemistry analyses demonstrated robust neurite development and prominent synapsin-positive puncta associated with neuronal connectivity and maturation. Neurobasal PhysioPlus Medium promoted substantially greater neurite outgrowth and neuronal persistence compared to another supplier's system, demonstrating the ability of the system to support challenging neuronal culture paradigms (Figure 3).

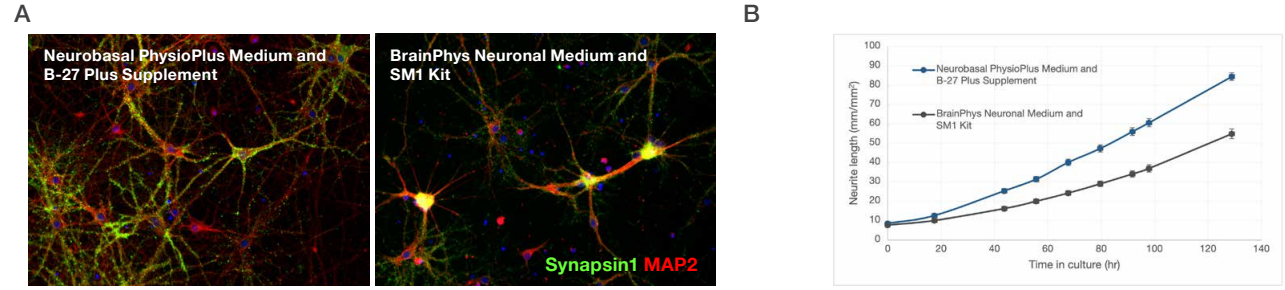

Figure 3. Morphological maturation of neurons in different culture systems. Rat cortical neurons (E18) were seeded at a low density (2.5×10^4 cells/cm^2) and maintained in Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement or with the BrainPhys Neuronal Medium and SM1 Kit for 14 days. **(A)** Cells were fixed and stained with Synapsin1 and MAP2 antibodies. The condition with Neurobasal PhysioPlus Medium shows high levels of Synapsin1-positive puncta across the MAP2-positive neurites. The BrainPhys and SM1 system shows culture degradation and significant aggregation of neurons. **(B)** Neurite outgrowth was quantified over the first 4 days in culture. The condition with Neurobasal PhysioPlus Medium showed a ~35% increase in neurite outgrowth compared to the BrainPhys and SM1 system.

Neurobasal PhysioPlus Medium promotes functional maturation at the single-cell level

To determine whether improvements in neuronal health and a formulation more similar to the extracellular environment of the CNS translated into functional maturation, neurons were evaluated using whole-cell patch-clamp electrophysiology (Figure 4).

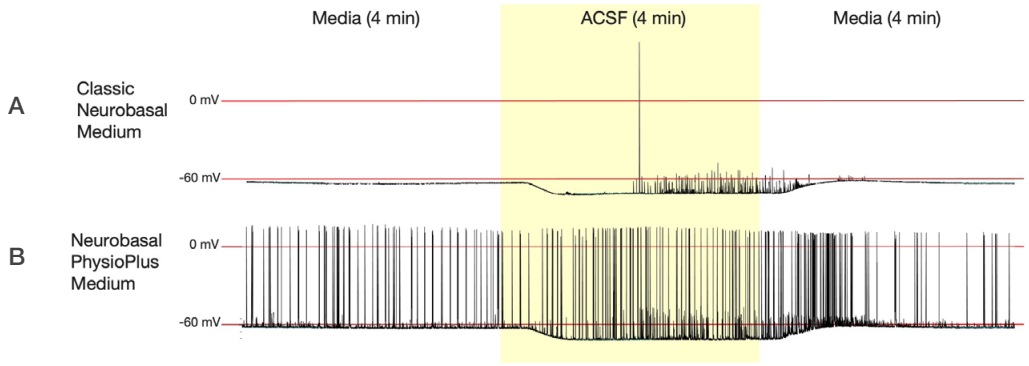

Figure 4. Differences in the electrophysiological properties of neurons observed by whole-cell patch-clamp recordings. Rat cortical neurons (E18) were plated on a confluent monolayer of rat astrocytes and cultured in **(A)** Gibco™ Neurobasal™ Medium supplemented with B-27™ Supplement or **(B)** Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement for 26 days. On day 26, whole-cell patch-clamp recordings were performed. Spontaneous action potential (AP) firing was examined while using the culture medium as the external recording solution and during exchange of the external solution with artificial cerebrospinal fluid (ACSF) to assess any gross differences in activity due to the external solution. Neurons grown in Neurobasal Medium exhibited minimal spontaneous activity in the presence of Neurobasal Medium but exhibited greater spontaneous activity in the presence of ACSF. Neurons grown in Neurobasal PhysioPlus Medium showed high levels of spontaneous action potential firing whether in Neurobasal PhysioPlus Medium or ACSF.

Neurons cultured in Neurobasal PhysioPlus Medium exhibited electrophysiological properties consistent with more mature and functionally competent neurons compared with cultures maintained in traditional Neurobasal Medium (Table 1). The observed properties included:

- Larger membrane capacitance
- Lower input resistance
- More hyperpolarized resting membrane potential
- Greater spontaneous firing activity
- Increased burst firing behavior

These characteristics are commonly associated with increased neuronal maturity and enhanced functional development.

Table 1. Interrogation of electrophysiologic properties of cells cultured and recorded in distinct culture systems.*

Parameter	Neurobasal PhysioPlus Medium	Neurobasal Medium
Capacitance	73 ± 6 pF (22)	44 ± 3 pF (29)
Input resistance	70 ± 8 MΩ (22)	92 ± 8 MΩ (29)
Resting membrane potential (RMP)	–60 ± 2 mV (22)	–50 ± 3 mV (29)
Percentage of neurons with a RMP below –50 mV	95% (21 of 22)	72% (21 of 29)
Number of spontaneous action potentials	26.9 ± 7.9 (20)	6.4 ± 5.9 (22)
Firing rate	0.15 ± 1.04 (20)	0.04 ± 0.03 (22)
Number of bursts	2 ± 1 (21)	0 ± 0 (22)
Percentage of neurons bursting	38% (8 of 21)	0% (0 of 22)
Rheobase	314 ± 40 pA (16)	152 ± 20 pA (23)

* Rat cortical neurons (E18) were plated on a confluent monolayer of rat astrocytes and were cultured in Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement or Neurobasal Medium with B-27 Supplement for 26 days. At day 26, whole-cell patch-clamp recordings were performed in voltage-clamp mode and current-clamp mode to measure various electrophysiologic properties of neurons cultured in the different medium systems. Values are presented as mean ± SEM (n), where SEM is the standard error of the mean and n is the number of neurons analyzed.

Enhanced spontaneous activity

A larger proportion of neurons cultured in Neurobasal PhysioPlus Medium displayed more spontaneous action potential firing compared with neurons cultured in conventional Neurobasal Medium. Increased spontaneous firing indicates the development of intrinsic neuronal excitability and functional neuronal networks.

Increased burst firing

Burst firing activity was observed more frequently in neurons cultured in Neurobasal PhysioPlus Medium. Burst firing is an important feature of mature neuronal communication and reflects improved neuronal network integration.

Electrophysiological properties consistent with maturation

Neurons cultured in Neurobasal PhysioPlus Medium exhibited higher rheobase values, larger membrane capacitance, and lower input resistance, consistent with larger and more mature neuron phenotypes.

Neurobasal PhysioPlus Medium accelerates network formation and supports long-term functional stability

Many neuroscience applications require not only healthy neurons, but also reproducible neuronal networks capable of generating stable activity over extended periods. Multi-electrode array (MEA) platforms help provide a powerful approach for assessing network development and functional performance (Figure 5).

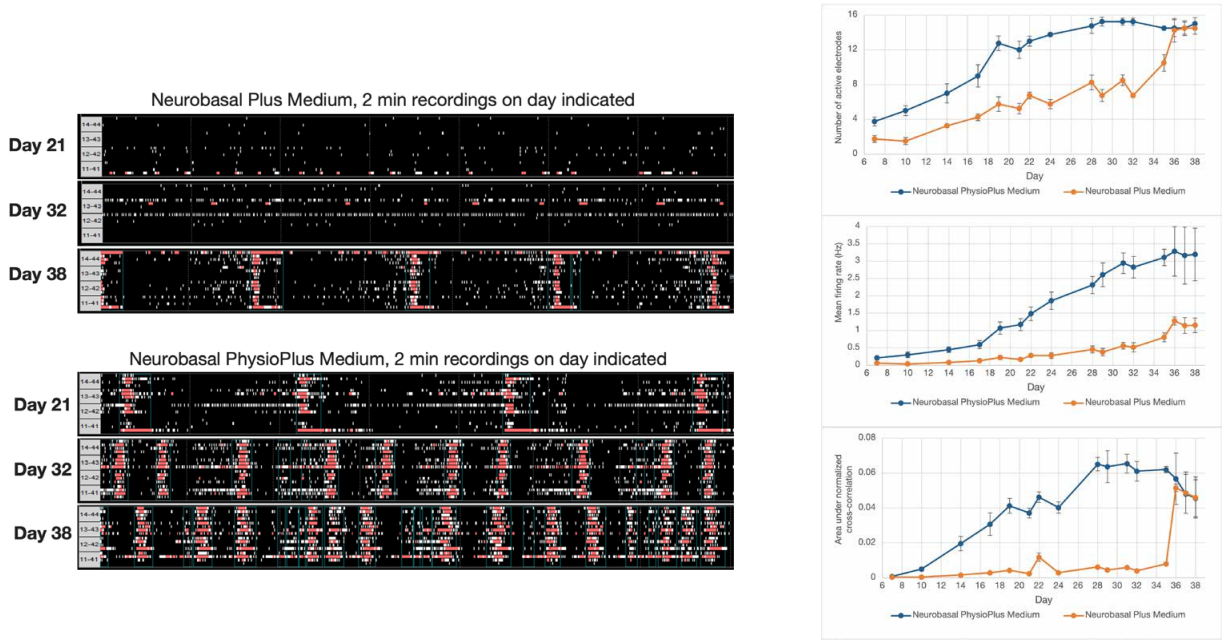

Figure 5. Neuronal activity and network formation assay (MEA) with PSC (rosette)-derived neurons. Rosette-derived progenitors were seeded (1.0 x 10^5 cells per 8 µL droplet per well) on MEA plates in differentiation medium for 5 days. After 5 days, medium was changed to Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement or Gibco™ Neurobasal™ Plus Medium with B-27 Plus Supplement. No growth factors were added to either condition. Spontaneous activity parameters were monitored for 38 days, including the number of active electrodes, mean firing rate (Hz), and synchrony (area under normalized cross-correlation). At day 38, both conditions had near equivalent levels of neuronal survival, yet the condition with Neurobasal PhysioPlus Medium with B-27 Plus Supplement showed improved spontaneous activity characteristics for each parameter measured.

Earlier onset of network activity

Neurons cultured in Neurobasal PhysioPlus Medium demonstrated an earlier emergence of spontaneous neural activity and network activity than neurons cultured in Neurobasal Plus Medium, which also supports exceptional cell survival. Functional activity appeared sooner and increased more rapidly during neuronal maturation.

Stronger network activity

Across the culture period, Neurobasal PhysioPlus Medium consistently supported higher mean firing rates and a greater number of active electrodes. These results indicate an increased recruitment of neurons into active networks and enhanced functional connectivity.

Sustained activity over time

Importantly, neuronal activity remained robust throughout extended culture periods. Rather than exhibiting transient peaks followed by declines, cultures maintained in Neurobasal PhysioPlus Medium displayed strong and sustained activity over time, supporting long-term experimental workflows.

Benchmarking MEA performance in different media systems

MEA assays were used to examine additional iPSC-derived neuron models that were cultured in different media systems in order to better understand how the media system impacts functional performance. ioGlutamatergic neurons, cultured alone or in combination with ioGABAergic neurons, revealed stark differences in the development of neural activity when cultured in the absence of growth factors (Figure 6). Cultures maintained in Neurobasal PhysioPlus Medium displayed activity at earlier timepoints in culture and greater activity at all timepoints throughout the culture.

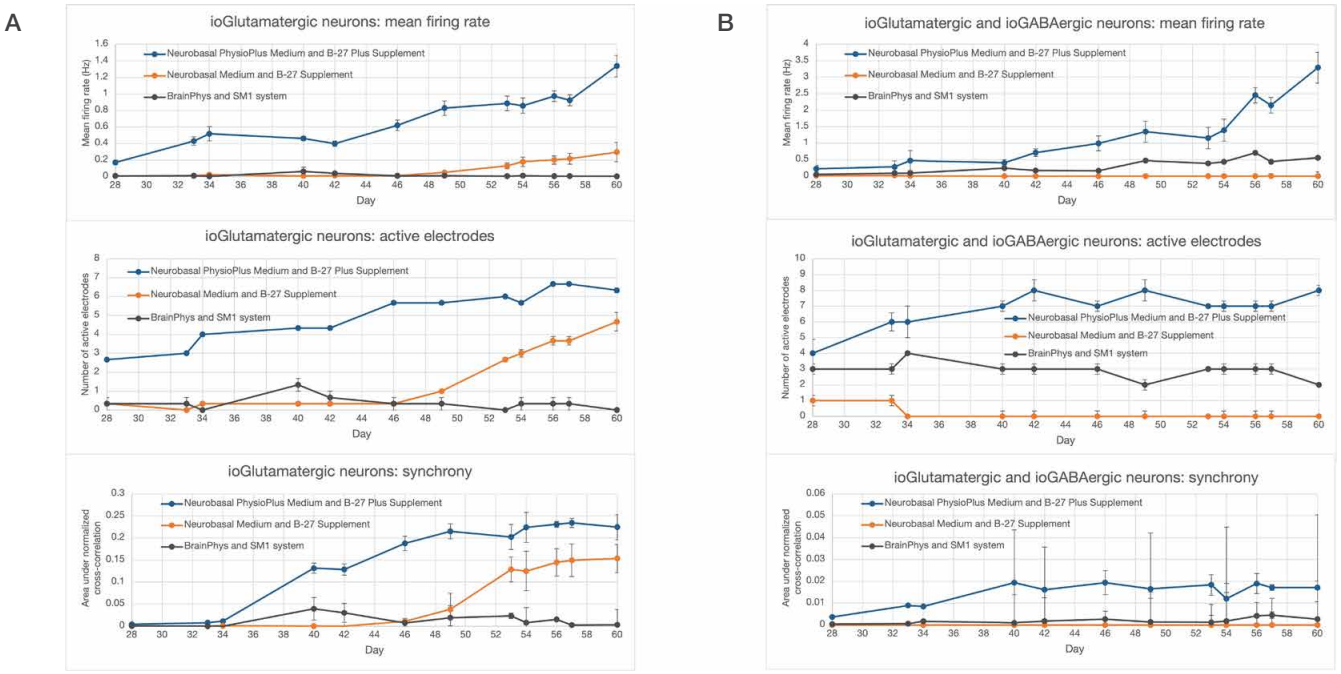

Figure 6. Neuronal activity and network formation assay (MEA) with induced PSC-derived neurons. (A) ioGlutamatergic or **(B)** ioGlutamatergic (70%) and ioGABAergic (30%) neurons from bit.bio were seeded on an MEA plate double-coated with poly-d-lysine and laminin, in neuronal induction medium for 5 days. After 5 days, medium was changed to Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement, original Neurobasal Medium with B-27 Supplement, or the BrainPhys Neuronal Medium and SM1 Kit. To assess the impact of neuronal culture systems on the development of neuronal activity and network activity, the recommended growth factors were not included in the medium after day 5. ioGlutamatergic neurons alone and 70% ioGlutamtergic neurons cocultured with 30% ioGABAergic neurons matured in Neurobasal PhysioPlus Medium over 60 days showed earlier onset and higher levels of neuronal activity (mean firing rate), increased number of active electrodes, and higher levels of network activity (synchrony), compared to neurons cultured in classic Neurobasal Medium with B-27 Supplement or with the BrainPhys Neuronal Medium and SM1 Kit.

Neurobasal PhysioPlus Medium supported similar performance in functional assays with primary rat cortical neurons (Figure 7). Neurobasal PhysioPlus Medium promoted early and prolonged maintenance of spontaneous neuronal activity compared to traditional neuronal cell culture media and, critically, the activity did not fall off during the 30 days of the experiment. Cultures maintained in Neurobasal PhysioPlus Medium also exhibited a higher proportion of electrically active neurons maintained across wells and plates. The network activity was also stable during long-term culture, increasing the useful experimental window of these neuronal cultures.

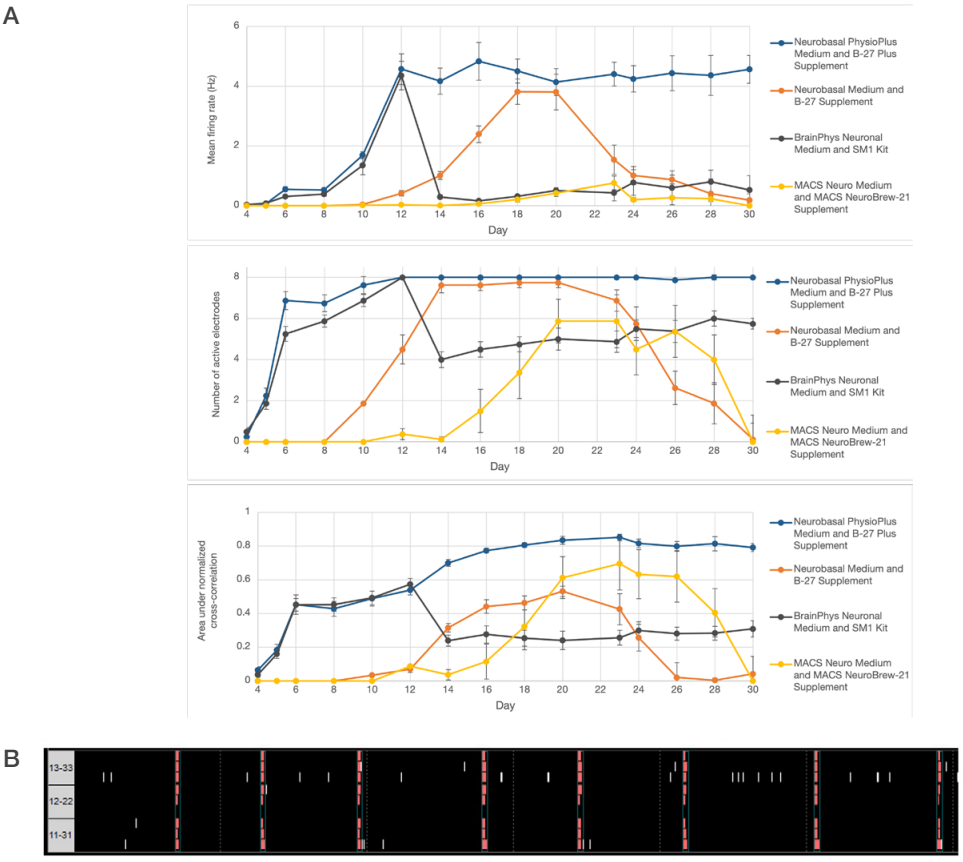

Figure 7. Neuronal activity and network formation assay (MEA) with primary neurons. Rat cortical neurons (E18) were plated and maintained in either Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement, classic Neurobasal Medium with B-27 Supplement, the BrainPhys Neuronal Medium and SM1 Kit, or MACS™ Neuro Medium supplemented with MACS™ NeuroBrew™-21 Supplement, for 30 days. Cells were seeded at 4 x 10^4 cells per cm^2 in an 8 µL droplet per well. **(A)** Neuronal and network activity parameters measured over 30 days for each culture system. **(B)** Example MEA raster plot of a 2-minute recording on day 21 from primary rat cortical neurons cultured in Neurobasal PhysioPlus Medium and B-27 Plus Supplement.

High-density MEA analysis demonstrates reliable network development

High-density MEA analysis performed by MaxWell Biosystems on MaxTwo Multi-Well High-Density Microelectrode Arrays (HD-MEAs) provided additional insight into network formation and stability (Figure 8). Multi-well HD-MEAs help enable precise recording and stimulation of electrogenic cells across multiple wells, combining single-cell sensitivity with scalable experimental capacity.

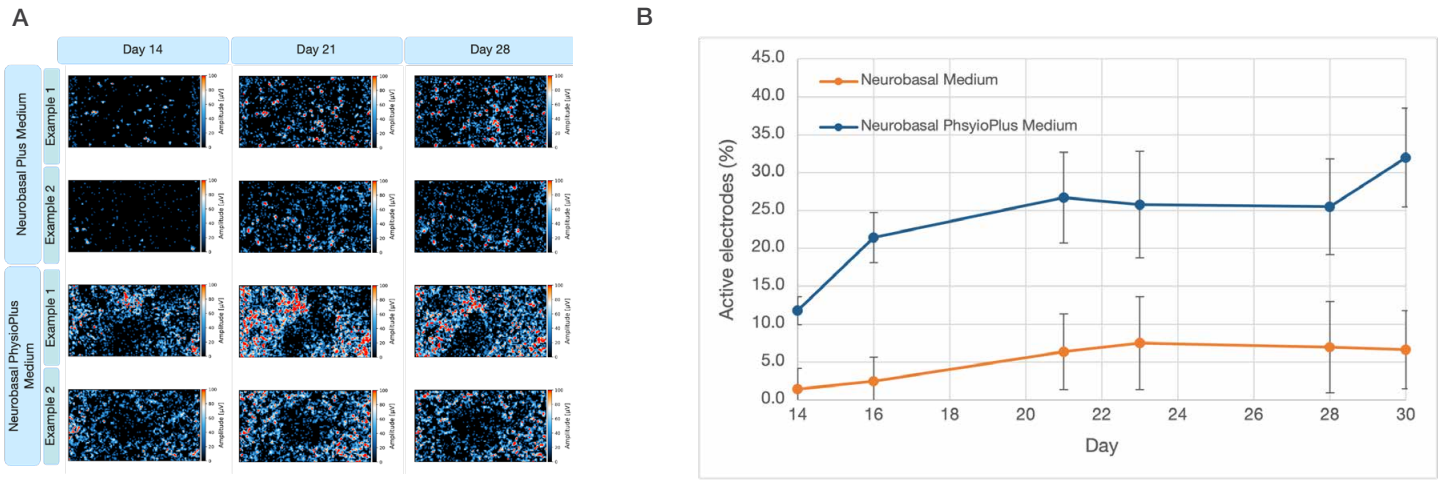

Figure 8. High-density MEA analysis of primary neurons. Primary rat cortical neurons (E18) were plated and maintained on high-density MEA chips in Neurobasal PhysioPlus Medium supplemented with B-27 Plus Supplement or Neurobasal Medium with B-27 Plus Supplement. **(A)** Representative active area heat maps are shown for two wells from each condition at three timepoints. The results show significantly higher active area, earlier onset of activity, and maintenance of consistent activity over three timepoints for the Neurobasal PhysioPlus Medium compared to Neurobasal Medium. **(B)** Percent active area plotted from three timepoints over 16 days shows ~3.5 times higher percent active area that is maintained across timepoints in Neurobasal PhysioPlus Medium compared to Neurobasal Medium.

Increased network recruitment

Neurobasal PhysioPlus Medium increased the active area of neuronal cultures, indicating recruitment of a larger proportion of neurons into functional networks. Greater recruitment can improve assay robustness and increase confidence in downstream analyses.

Faster attainment of stable network states

Neuronal cultures reached mature network behavior more rapidly when maintained in Neurobasal PhysioPlus Medium. Earlier stabilization of network activity can shorten assay development timelines and expand the experimental window available for pharmacological studies.

Stable network dynamics

Network bursting parameters remained highly consistent over time, with reduced variability across measurements. Inter-burst interval (IBI) coefficient of variation (CV) analyses demonstrated stable network pacing and reproducible neuronal activity patterns (Figure 9).

Together, these results suggest that Neurobasal PhysioPlus Medium supports the development of reliable neuronal networks well suited for longitudinal studies and compound screening applications.

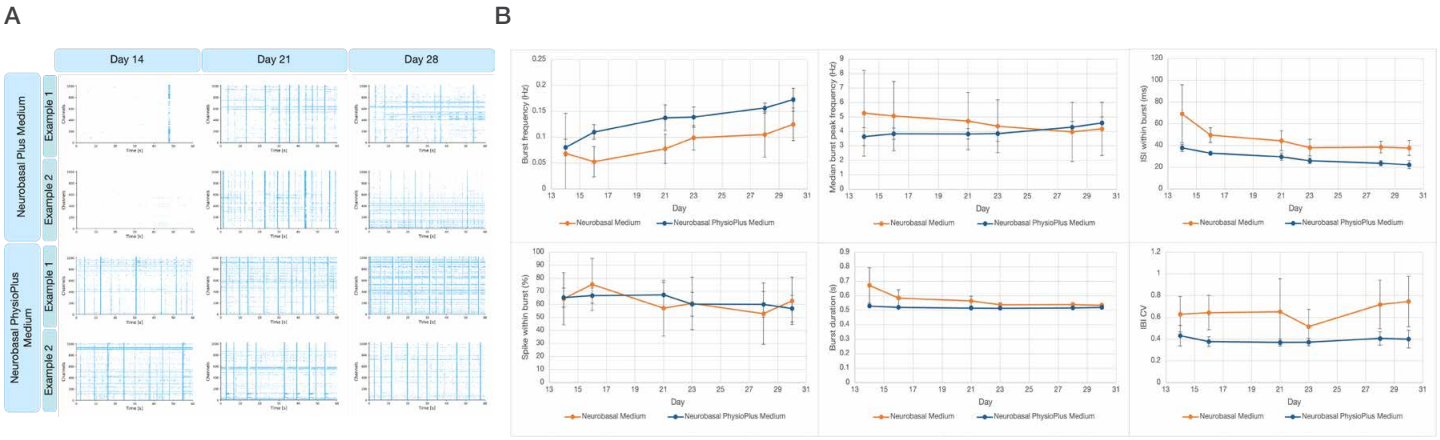

Figure 9. Evaluation of network bursting parameters. Primary rat cortical neurons (E18) were plated and maintained on high-density MEA chips in Neurobasal PhysioPlus Medium with B-27 Plus Supplement or Neurobasal Medium with B-27 Plus Supplement. **(A)** Representative raster plots of 1,000 recording electrodes are shown for two wells from each condition at three timepoints. The results show early onset of stable and consistent network bursting over three timepoints for the Neurobasal PhysioPlus Medium compared to later onset and highly variable bursting in the Neurobasal Medium. **(B)** Multiple parameters were plotted at six timepoints, including burst frequency, median burst peak frequency, inter-spike interval (ISI) within burst, spikes within burst, burst durations, and inter-burst interval coefficient of variation (IBI CV).

Conclusions

Creating predictive neuronal models requires culture systems that support both physiological relevance and reliable functional performance.

Neurobasal PhysioPlus Medium was developed to better reflect the extracellular environment experienced by neurons *in vivo* while maintaining the exceptional neuronal health enabled by B-27 Plus Supplement. Across primary and hiPSC-derived neuronal models, the Neurobasal PhysioPlus Medium and B-27 Plus Supplement system supported:

- Excellent neuronal survival and long-term maintenance
- Enhanced neurite development and maturation
- Electrophysiological properties consistent with mature neurons
- Earlier onset of spontaneous activity
- Stronger and more sustained network function
- Stable neuronal network behavior suitable for advanced functional assays

By combining physiological design principles with robust neuronal support, Neurobasal PhysioPlus Medium and B-27 Plus Supplement help provide researchers with a neuronal culture system capable of generating more relevant and reliable *in vitro* neuronal models.

Learn more at **thermofisher.com/order/catalog/product/A40008171**

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