

IMPROVED SURVIVAL, MATURATION, AND ELECTROPHYSIOLOGY OF PRIMARY NEURONS WITH THE B-27™ PLUS NEURONAL CULTURE SYSTEM

Michael Derr, Jonathan Sagal, Binjun Liu, Richard Josephson, Yan Yiping, Navjot Kaur and David Kuninger
Division of Cell biology, Thermo Fisher Scientific Inc., 7335 Executive Way, Frederick, MD 21704

INTRODUCTION

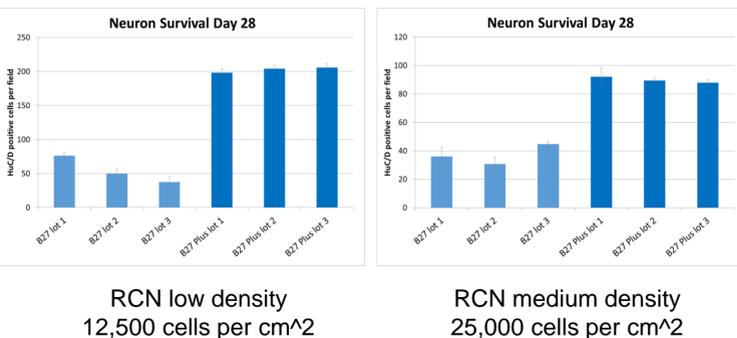
For over 25 years, the classic Gibco™ B-27™ Supplement and Gibco™ Neurobasal™ Medium have set the standard in neuronal cell culture reagents. The Gibco™ B-27™ Plus Neuronal Culture System, composed of the Gibco™ B-27™ Plus Supplement and Gibco™ Neurobasal™ Plus Medium, improves upon the classic culture environment through raw material and manufacturing upgrades and minor formulation modifications. Together, these small changes yield big results.

Primary rodent neurons are valuable model systems for studying a wide range of processes in neurobiology including development, disease, and toxicity. 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. The labor intensive nature of primary rodent neurons further highlights the importance of consistent and robust culture conditions.

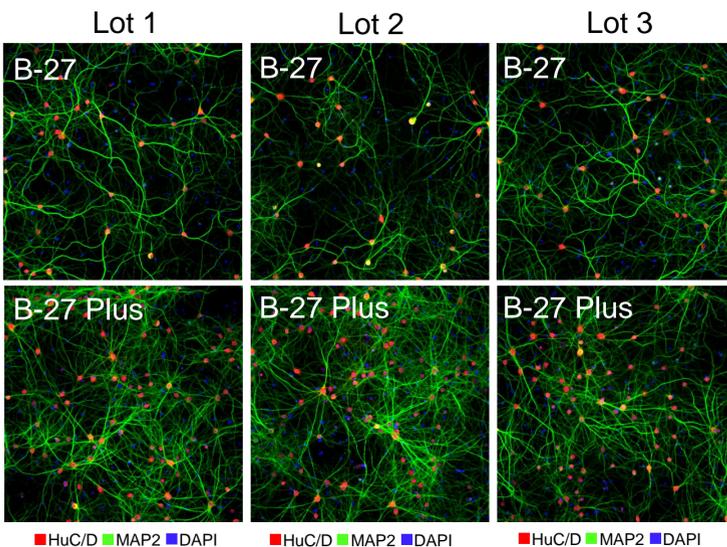
The key benefits of the B-27™ Plus neuronal culture system include increased neuronal survival, maturation, and functional activity. Here we use a range of analysis tools to demonstrate the benefits of the B-27™ Plus system compared to classic B-27™ medium. In addition, we look at lot to lot consistency in the context of neuronal survival to demonstrate the results of improvements made to the manufacturing process.

Figure 1. B-27™ Plus neuronal culture system: neuronal survival and B-27™ Plus Supplement lot testing

a) Neuronal survival quantitation: Rat cortical neurons 28 days in culture

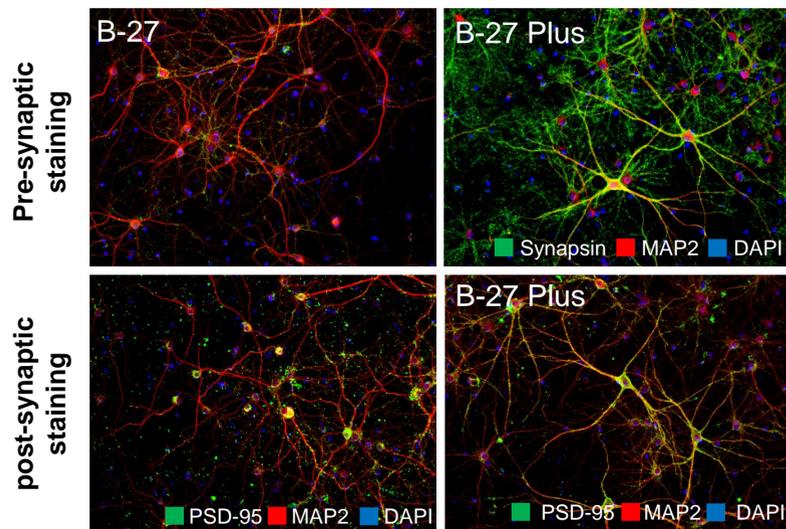


a) Neuronal survival and morphology: Rat cortical neurons 28 days in culture



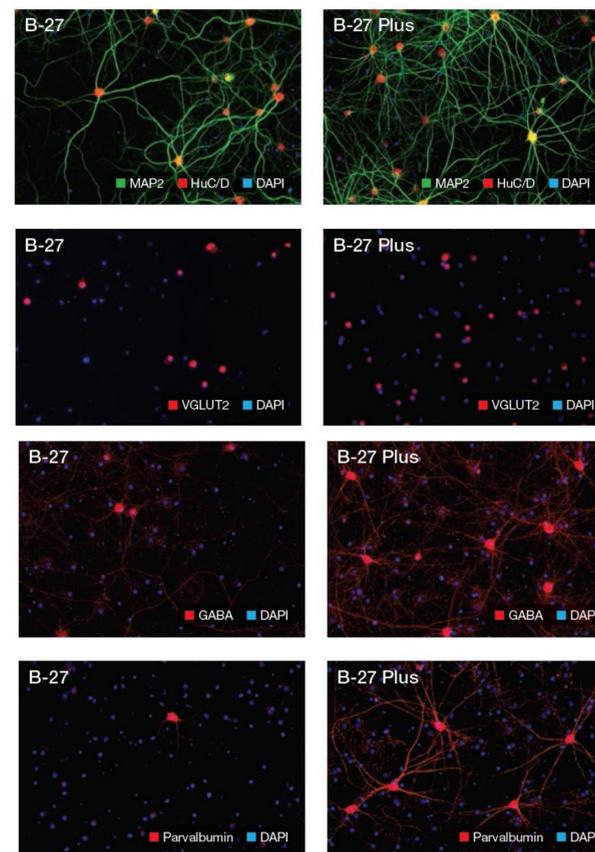
Images captured on CellInsight CX7 High Content Analysis (HCA) Platform

Figure 2. Enhanced maturation: pre and post-synaptic marker expression



Rat cortical neurons cultured for 28 days

Figure 3. Maintenance of neuronal subgroups

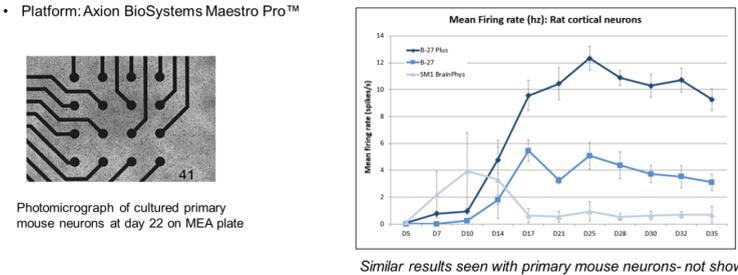


- Relative percent of excitatory (glutamatergic, VGLUT2+) neurons similar: 90% for “classic and 81% B-27 Plus
- Relative percent of inhibitory (GABAergic, GABA+) similar : 12% for “classic and 14 % B-27 Plus
- Relative percent of parvalbumin positive interneurons (subset of GABAergic) was DIFFERENT : less than 1% for “classic compared to 7 % B-27 Plus

PV interneurons play a critical role in controlling synchronous activity across networks of excitatory and inhibitory neurons

Figure 4. Improved functionality

- Multi-electrode array (MEA) analysis to assess “spontaneous” activity
- Platform: Axion BioSystems Maestro Pro™

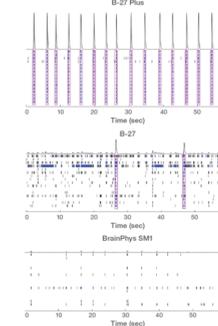
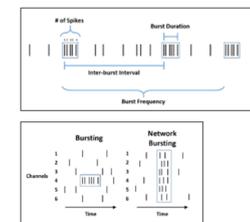


Photomicrograph of cultured primary mouse neurons at day 22 on MEA plate

Similar results seen with primary mouse neurons- not shown

B-27™ Plus Neuronal Culture System enables much higher and sustained electrophysiology activity than the classic B-27™ system or leading supplier’s medium.

MEA 101: Spontaneous activity parameters- mean firing rate, bursting, network bursting



- Raster Plots of synchronized bursting in day 28 RCNs
- Spiked traces indicate calculated bursts of activity
- B27™ Plus system supports highly synchronous network activity

CONCLUSIONS

1. Improvements to manufacturing process and raw material screening increase lot to lot consistency
2. Over 50% higher survival of primary neurons compared to the classic B-27™ system
3. Enhanced maturation as seen by increased levels of pre and post-synaptic markers
4. Maintenance of neuronal subgroups
5. Improved functional activity

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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