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Development of an Advanced Media System for Improved Neuronal Viability and Function

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ABSTRACT

Neuronal cell cultures offer an indispensable tool for investigating fundamental questions in neurobiology and applied applications in cell therapy and drug discovery. As such, the ability to establish and maintain primary neuronal cultures is essential for the comprehensive study of neuronal development and function. Neurons are usually cultured in serum-free systems which include a basal medium and supplements such as Neurobasal™ medium supplemented with B-27™ respectively. Here, we describe a new serum free neuronal media system that provides significant improvements for long term viability and functionality of primary and PSC-derived neurons *in vitro*. This new system, comprising a neuronal basal medium (Neurobasal™ Plus) and serum-free supplement (B-27™ Plus), is specifically optimized for the maturation and viability of primary rat, mouse and human PSC-derived neurons for long term cultures at both low and high cell density. Performance was evaluated by a number of criteria including neurite outgrowth, viability, relative purity and functionality. Typically cultures were assessed at 7, 14 and 21 days following plating primary neurons or addition of maturation medium to iPSC-derived neurons. The cultures were assessed for cell survival and neurite outgrowth by quantitative morphometric analysis using the Incucyte® Zoom live cell imaging system. Neuronal cell numbers (viability over time) was assessed by quantitative immunocytochemistry (ICC) using HuC/HuD to demarcate neuronal populations. HuC/HuD staining is localized to the cell body of neurons, facilitating quantification and throughput using an automated high content analysis system. In addition, primary rat neurons cultured in Neurobasal ™ Plus and B-27™ Plus system showed higher maturity showing superior levels of expression of synaptic markers Synapsin and MAP2 by ICC and produced higher spike rates on multielectrode arrays (MEA). Taken together these results demonstrate that our new B-27™ Plus Supplement and Neurobasal™ Plus Medium culture system is a superior solution to the current trusted standards used for culturing primary neurons, and maturing and maintaining hPSC-derived neurons.

MATERIALS AND METHODS

Recovery and Culturing of Primary Neurons: Cryopreserved Rat cortex Neurons (Cat # A10840), Rat Hippocampal Neurons (Cat # 10841), Mouse Cortex (Cat # A15585) or Mouse hippocampal neurons (Cat # A15587) were thawed quickly in a 37ºC water bath, diluted in pre-warmed media and the percentage viability was measured by Trypan blue assay. The cells were cultured in Neurobasal™ medium (Cat # 21103)or Neurobasal™ Plus (Cat # A3582901) supplemented with 2% B-27® (Cat # 17504) or B-27 Plus™ (Cat # A3582801) and 0.5 mM GlutaMAX™-I (Cat # 35050) and plated on poly-D-lysine coated dishes. Neurons were plated at 6×10^4 viable cells/cm^2 unless otherwise stated.

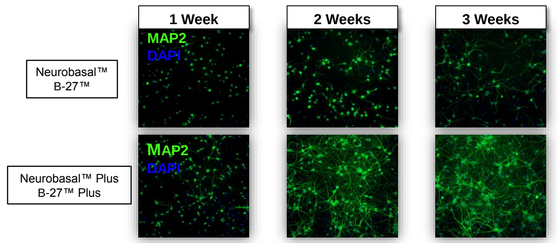
Culturing of NSC-derived Neurons: Neural stem cells (NSCs) were derived from H9 human embryonic stem cells (ESCs) by Gibco™ PSC Neural Induction Medium. Expanded NSCs were plated on Poly-D-lysine and laminin double coated 96-well plate at 2.5x10^4 cells/cm^2 and treated with CultureOne™ supplement. Human fetal-derived neural stem cells (NSCs) were plated on Geltrex-coated 48-well plate at 5x10^4 cells/cm^2.

Neuronal Survival: Cells were cultured for up to 3 weeks, fixed with 4% paraformaldehyde and permeabilized with 0.3% Triton-X at week 1, 2 and 3. Cells were stained with anti-HuC/D (Cat # A21271 antibody and counter stained with DAPI. The number of HuC/D positive cells were quantified with CellInsight™ CX5 High Content Screening . The data represents the average number of HuC/D positive cells per field from at least three wells (n=3), six fields were captured per well.

Neurite Outgrowth: Plates were scanned at various time intervals using Essen Incucyte®. Neurite length was quantitated from phase contrast images by applying NeuroTrack analysis tool.

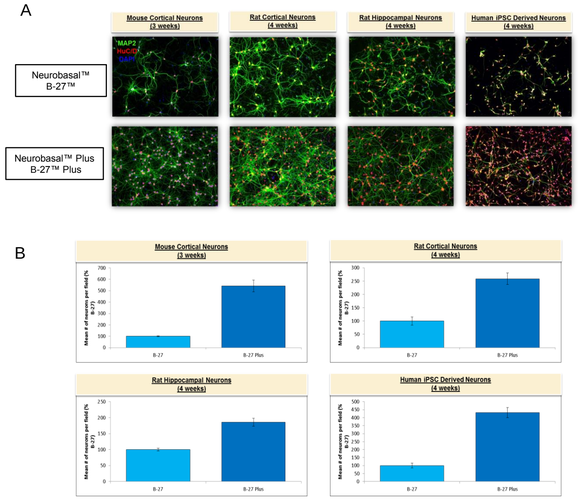
Multi Electrode Array (MEA): Neurons were plated on to 48 well MEA plates. Cells were cultured for 35 days in specified media conditions following the supplier's recommended protocols. Spontaneous electrophysiological activity was recorded throughout with the Maestro MEA platform (Axion BioSystems).

Figure 1. Gibco™ B-27™ Plus Neuronal Culture System increases neuronal survival



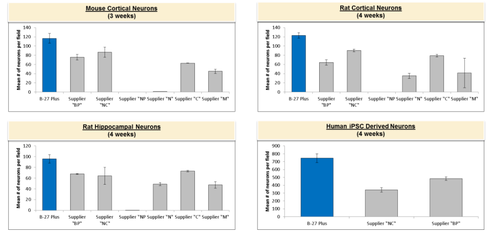
Primary rat cortical Neurons were maintained for 3 weeks in classic system containing B-27™ /Neurobasal™ or new B-27™ Plus Neuronal Culture system containing B-27™Plus and Neurobasal™ Plus. Neuronal survival was measured by immunofluorescent labeling of MAP2, performed on days 7, 14 and 21. Neurons maintained a significantly higher number of neurons at each time point in the B-27™ Plus Neuronal Culture System showing higher neuronal survival in short and long term cultures.

Figure 2. B-27™ Neuronal culture system provides superior culture system for primary rodent and human iPSC derived neurons



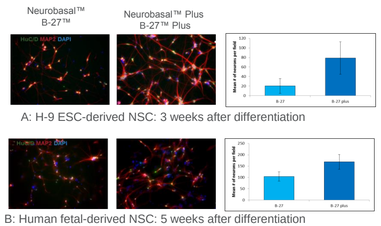
(A) Cryopreserved neurons were cultured for 3 – 4 weeks in the listed media system. Neurons were immuno-stained with neuronal dendritic marker, MAP2 (green), neuronal cell body marker, HuC/D (red), and nuclei were counter-stained with DAPI (blue). Immunostaining was performed at indicated time points. (B) For quantitative image analysis, HCS Studio image analysis software was used to count the number of neurons (HuC/D positive cells). The data represents the average number of HuC/D positive cells per field from three wells (n=3), six fields were captured per well. Data shown from one of the n ≥ 3 experiments. Comparability studies indicates that the B-27™ Plus Neuronal Culture System is a significantly superior media compared to the classic B-27™ supplemented Neurobasal™, with improved neuronal survival.

Figure 3. B-27™ Plus Neuronal Culture System enables highest survival of neurons compared to alternative media systems



The neurons were maintained for 3 – 4 weeks in B-27™ Plus and alternative serum-free supplemented media systems following the supplier's recommended protocols. Neuronal survival was quantitated by immunofluorescent labeling of neuronal cell body marker HuC/D. Data shown are from one of three experiments, with each run showing that B-27 Plus™ enables the highest neuronal survival among the alternative commercial systems.

Figure 4. B-27™ Plus Neuronal culture system improves differentiation of NSC to neurons

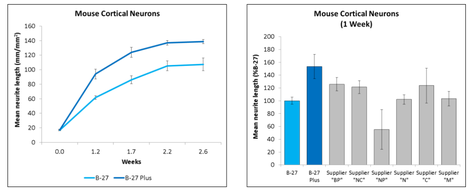


A: H-9 ESC-derived NSC: 3 weeks after differentiation

B: Human fetal-derived NSC: 5 weeks after differentiation

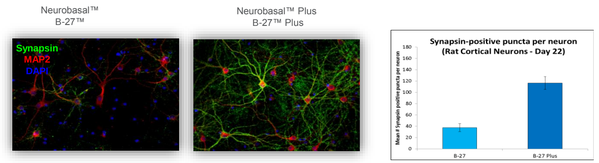
(A) Neural stem cells (NSCs) derived from H9 human embryonic stem cells (ESCs) were cultured in Neurobasal™ supplemented with B-27™ or B-27™ Plus Neuronal Culture System . Cells were fixed at 3 weeks after differentiation. (B) Human fetal-derived neural stem cells (NSCs) were cultured for 5 weeks after differentiation In bothe cases cells were stained with antibodies against HuC/D and MAP2. Cell nuclei were stained with DAPI. The number of Hu C/D positive cells were quantified with CellInsight™ CX5 High Content Screening Platform. B-27™ Plus Neuronal culture system showed improved survival of differentiated neurons from hPSC-derived NSCs and human fetal-derived NSCs.

Figure 5. B-27™ Plus Neuronal culture system enables improved neurite networks



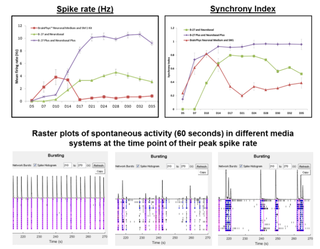
Primary mouse cortical neurons cultured in B-27™ Plus Neuronal Culture System showed accelerated and increase neurite outgrowth. Neurite length was quantitated using Essen Incucyte® from phase contrast images. Data shown are from one of three experiments, with each run showing that B-27 Plus™ enables the highest neuronal survival among the alternative systems.

Figure 6. B-27™ Plus Neuronal Culture System Enhances neuronal maturation



Primary rat cortical neurons were cultured for 22 days and stained with dendritic-mictotubule marker MAP2 and with Synapsin1/2 to label pre-synaptic terminals. The synaptic positive puncta per neurons were quantitated. B-27 Plus Neuronal Culture System improves neuronal maturity as demonstrated by a three-fold increase in Synapsin-positive puncta per neuron.

Figure 7. B-27™ Plus Neuronal Culture System Improves electrophysiological activity



Spontaneous activity from rat cortical neurons cultured in B-27™ or B-27™ Plus or BrainPhys was recorded at 2-4 days interval for 35 days. Data is shown from one of the three experiments . Neurons cultured in BrainPhys showed increase in spike activity at earlier time points and declined activity within a week, where as neurons cultured in B-27™ Plus system showed gradual increase over time and stayed active for long term. B-27 Plus System results in consistent, stable, and highly synchronized spontaneous activity over time. This enables a low background system with increased sensitivity and simplified analysis of MEA data.

CONCLUSIONS

B-27™ Neuronal culture system:

- Enables the highest survival of primary and human stem cell derived neurons.
- Accelerates and improves neurite outgrowth , maturation, and functionality
- Seamlessly replaces current media used to maintain, mature, or differentiate neurons.

ORDERING INFORMATION

- B-27 ™ Plus Neuronal Culture System (supplement and Medium): Cat. No. A3653401
- B-27™ Plus Supplement (50X), 10mL- Cat. No. A3582801
- Neurobasal ™ Plus, 500mL –Cat. No. A3582901

TRADEMARKS/LICENSING

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