Optimized neuronal culture media for Electrophysiology

life technologies™

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ABSTRACT

Primary neural cells offer an indispensible tool for dissecting basic cell biology with prospective applications in cell therapy and drug discovery. Utilization of their potential largely relies on optimal culture conditions that preserves it functional properties. In particular, electrophysiological properties of neurons are known to diminish under suboptimal culture conditions. It is through electrical activity that neurons communicate with each other as well as with muscle and other end organs. Electrophysiology is the fundamental technique to assess such electrical activities to study the functions and dysfunctions in cultured neurons. Neurons are usually cultured in serum-free systems which include basal medium such as Neurobasal® supplemented with B-27® and GlutaMAX-I®. Neurons cultured in such media show lower electrophysiological spike rates relative to media supplemented with serum. We have developed an Electrophysiology kit components- Neurobasal® Electro and B-27® Electro which promote higher spike rates by a mechanism involving greater sysnoptogenesis that is reflected by increased immunocytochemical marker expression of pre-synaptic Synaptophysin and post synaptic PSD-95. The immunoreactive GABA and NMDA puncta also increased 2.8-fold and 1.6-fold respectively (n=3, p<0.05) over the period of three week. Primary rat neurons cultured in Neurobasal Electro® and B-27® Electro produced higher spike rates on multielectrode arrays. The rate of cell survival was indistinguishable at day 4 between Neurobasal®/B-27® and B-27® Electrophysiology kit assessed by Live/Dead® cell viability assay (n=3, 98.0 ± 0.8 vs. 97.2 ± 1.6). These results show that this media system is an improvement to Neurobasal®/B-27® for cultured networks with an increased density of synapses and transmitter receptors. B-27® Electrophysiology kit components-Neurobasal Electro® and B-27® Electro will render better electrophysiological studies, besides normal functional studies enabling improved screening applications.

MATERIALS AND METHODS

Primary Neuronal Cultures: Rat E18 freshly isolated or cryopreserved cortical or hippocampal neurons were cultured on poly-D-lysine coated surfaces. Cells were maintained in Neurobasal® medium supplemented with 2% B-27® or Neurobasal®, Electro medium supplemented with B-27®, Electro. Both media were further supplemented with 0.5 mM GlutaMAX™-I for 4 to 21 days in standard 5% CO_2 conditions.

Measurement of electrical activities: Rat hippocampal neurons were plated at 1000 cells/mm² onto a multi electrode array Axion Biosystems MEA system (MM Muse 01) and spike rates were calculated using accompanying software. Cells were allowed to cultured in each media and readings were acquired on day 7, 14, and 21. Statistical analyses used student *t*- test or ANOVA with rejection of the null hypothesis at p<0.05.

Cell survival assessment: Cells were stained using Calcein-AM and EthD1 to evaluate the survival rate of neurons using LIVE/DEAD cell viability /cytotoxicity kit. Live (green) and dead (red) cells were counted using ImageJ and cell survival was calculated as percentage of live cells over total cell population. Statistical analyses used student *t*- test or ANOVA with rejection of the null hypothesis at p<0.05.

Immunocytochemistry: Cells were cultured for 4 to 21 days and fixed in 4% paraformaldehyde, permeabilized with 0.3% Triton® -X, and blocked with 5% goat serum. The cells were incubated with primary antibodies for overnight and with secondary antibodies for one hour at room temperature. Antibodies details are provided in figure legends.

RESULTS

Figure 1. Measurement of electrical activity

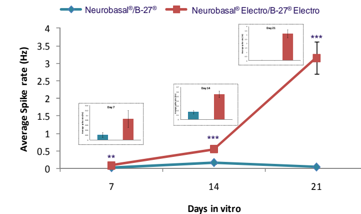

Fig 1: Multielectrode array to measure spontaneous spike rates: Hippocampal neurons cultured in B-27® Electrophysiology kit produced higher spike rates as compared to neurons cultured in Neurobasal® medium supplemented with 2% B-27®. Day 7=4.2, day 14=3.4 fold and day 21= 78 fold increase; n=2, ** denotes $p < 0.001$, *** denotes $p<0.0001$.

Figure 2. Comparison of cell survival

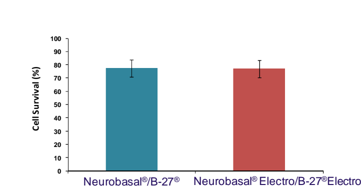

Fig 2: Live/Dead® Viability/Cytotoxicity assay: Neurons cultured in B-27® Electrophysiology kit maintain equivalent cell survival rate as compared to neurons cultured in Neurobasal® medium supplemented with 2% B-27®. Neuronal survival rate was comparable even at day 21 cultures (n=5, 77± 6 vs.78 ±7).

Figure 3. Neurite Outgrowth

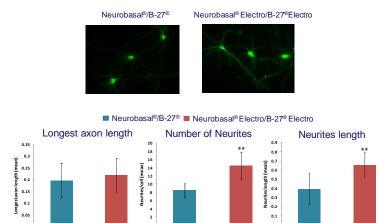

Fig 3: Comparison of neuronal branching in B-27® Electrophysiology kit *vs.* Neurobasal® /B-27® : Rat cortical neurons showed 1.7 fold increase in number of neurites per cell, and 1.6 fold increase in dendrite length The means of longest axon length were not significantly different. (n=10 fields, ** denotes $p < 0.001$).

Figure 4. Neurotransmitter receptors expression

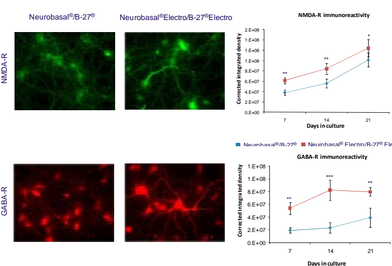

Fig 4: Rat cortical neuron cultures were immunostained with anti-NMDA-R, (green) and anti-GABA-R (red) antibodies. Neurons cultured in B-27® Electrophysiology kit produced 1.6-, 1.5-, 1.2-fold increase in NMDA receptor and 2.8-, 3.5-, 2-fold increase in GABA receptor immunoreactivity at day 7, 14, and 21 respectively. n=2,
* denotes $p < 0.05$, ** $p < 0.001$, *** $p<0.0001$.

Figure 5. Synaptogenesis comparison

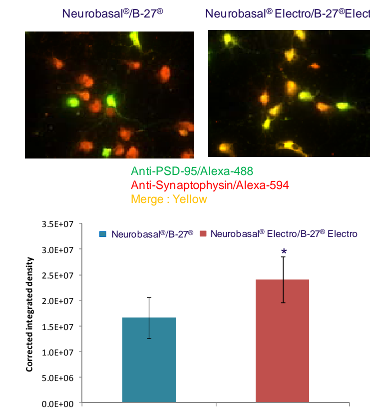

Fig 5: Rat cortical Neurons cultured in B-27® Electrophysiology kit showed higher density of synapses. Neurons were immunostained for pre-synaptic vesicle marker Synaptophysin (red) and post-synaptic marker PSD-95 (green) and images were merged (yellow) shown in upper panel. Integrated density was measured using ImageJ resulting in 1.5-fold increase in receptors expression measured from merged images. n=5 fields,
* denotes $p < 0.05$.

CONCLUSIONS

1.The B-27® Electrophysiology Kit is specifically designed to enhance electrophysiological spike rates in neural cell cultures when compared with other media and supplement combinations.

2.The complete medium is prepared by combining kit components Neurobasal® Electro with 2% B-27® Electro and additional 0.5mM GlutaMAX™-I.

3.Hippocampal and cortical neurons cultured in this new formulation show higher density of synapses, and neurotransmitter receptors.

4.B-27® Electrophysiology kit show greater neurite branching.

5.B-27® Electrophysiology kit promotes optimal viability and long-term survival of rat embryonic hippocampal and cortical neurons.

6.Neurons cultured in this system improve the efficiency of multielectrode arrays used in neurotoxicology and neuropharmacology studies.

REFERENCES

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4.Das, M. *et al.* (2010) A Defined Long-Term In Vitro Tissue Engineered Model of Neuromuscular Junctions. Biomaterials. 31(18): 4880–4888.

PRODUCT INFORMATION

B-27® Electrophysiology Kit	**A1413701**	**1kit**
The kit contains:		
B-27® Supplement, Electro (50X)		10mL
Neurobasal® Medium, Electro (1X)		500mL

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