MTI-GlobalStem

Application Note

Primary neurons are widely used to study synaptic function, morphology, neurotoxicity, neurotransmitter release and disease modeling.

They enable researchers to more closely explore neuronal activity than would be possible in animal models.

Culturing primary neurons long-term is notoriously difficult.

One major problem associated with cultured neuronal networks is their short survival.

Susceptibility to infection, hyperosmolality related to medium evaporation and the fact that it takes several weeks to months to observe network-wide activity makes increasing viability and extending neural life span *in vitro* extremely important.

Sensitivity of Primary Neurons in Culture

Using the Right Combination of Media, Supplements and Neural Substrates

Growing and maintaining primary neurons long-term in culture requires excellent cell-culture handling skills along with an optimal combination of media, serum-free supplements and substrates. Paying close attention to these culture conditions is important to ensuring your primary neurons' survival. Here we answer some of your questions about culturing healthy primary neurons, and offer tips to make the process as efficient as possible.

Questions & Answers

Q1. I notice that when I culture my primary neurons in 96- or 384-well plates, the media in the outer wells is much lower than in the inner wells of the plates. How can I prevent this?

A1: When plating in 96- or 384-well plates, evaporation in the outer wells can occur, causing edge effects that can lead to variability in experimental results. One way to minimize edge effects is to use a thin membrane of fluorinated ethylene propylene (such as FEPTeflon® RFilm from American Durafilm, Holliston, Mass.). This thin membrane can be placed between the lid and the plate and is permeable to oxygen, yet contains no pores and thus retains water vapor while blocking microbes. You should observe better consistency across your plate if you use FEP in your primary culture protocol.

Q2. I am trying to grow my primary neuronal cultures beyond 14 days, but as they approach day 21, they begin to die off. Is there anything I can do to increase long-term viability?

A2: It is important that primary neurons receive proper nutrients throughout their long-term culture. One recommendation is to do half-media changes every three or four days to prevent nutrient loss. If that doesn't work, check

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your supplements. Culturing neurons without serum requires the use of a defined supplement, but these vary wildly in their ability to support neurons in culture. Finding reagents that use high-quality raw materials is extremely important.

One such reagent is GS21™ Supplement, a defined neural supplement that improves long-term viability in primary neurons while ensuring lot-to-lot consistency. This supplement will improve neuronal maturation and neurite outgrowth and increase long-term viability.

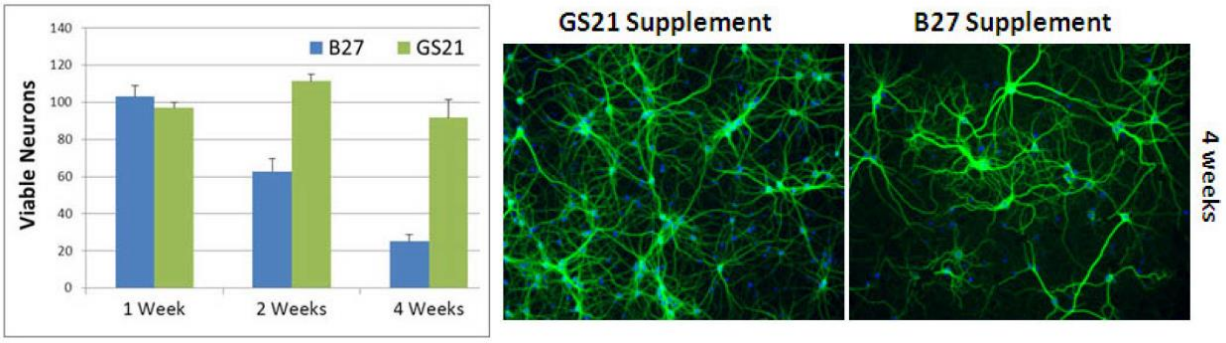

Figure 1. Primary neurons cultured in GS21™ (MTI-GlobalStem) supplemented media show significantly improves neuron viability after 2 weeks in culture (p<0.05; image not shown) and 4 weeks (p<0.05; image on right) in culture, as compared to cells cultured in B27® supplement from Life Technologies.

Q3. After about two weeks of culture, my primary neurons begin to detach from the wells. I have checked all the reagents, and they seem to be fine. I am using poly-L-lysine for cell attachment. Could that be the problem?

A3: Morphology, differentiation, adhesion and growth are greatly affected by the type of growth substrate used when culturing cells. Neurons, because of their unique morphology, need something suitable for the cells to attach to. Central nervous system (CNS) neurons tend to adhere nicely to polyornithine or poly-D-lysine, which are polymers of basic amino acids, as well as to laminin, a glycoprotein. When using poly-L-lysine, try using a formulation with a higher molecular weight—the shorter the polymer, the more toxic it can be to your neurons, possibly causing them to detach. Poly-ornithine, poly-D-lysine and laminin, when used in combination with a serum-free basal medium and serum-free supplement, have been shown to improve the overall growth and performance of primary neurons.

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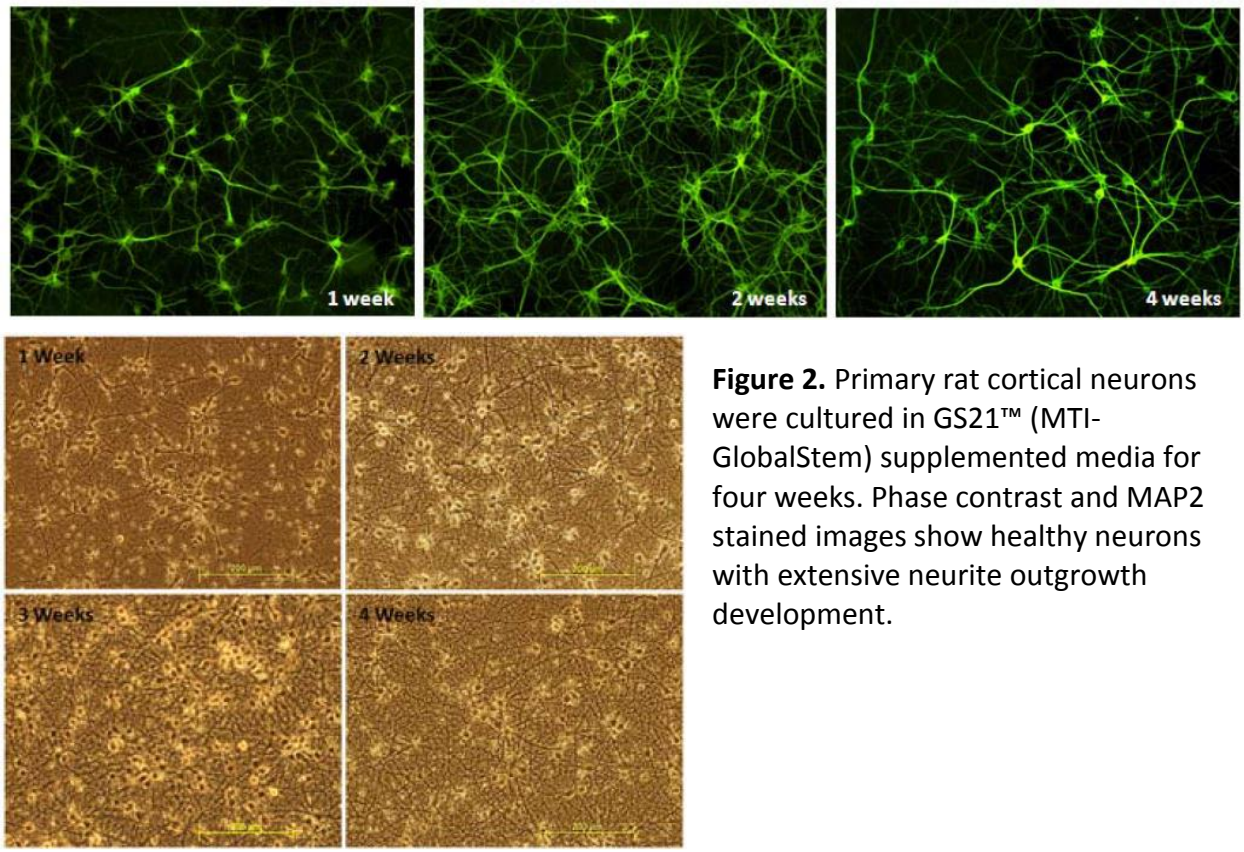

Figure 2. Primary rat cortical neurons were cultured in GS21™ (MTI-GlobalStem) supplemented media for four weeks. Phase contrast and MAP2 stained images show healthy neurons with extensive neurite outgrowth development.

Growing primary neurons long-term *in vitro* can sometimes prove to be a daunting task especially when your cells are highly susceptible to infection and hyperosmolarity due to medium evaporation. Paying close attention to culture conditions is important to ensuring that your primary neurons maintain lasting survival.

To ensure success when culturing your primary neurons understand that culture conditions are crucial to maintaining neuronal health. Be consistent with your operational procedures, making sure to create a detailed protocol that you can routinely follow. After plating your neurons, let them sit at room temperature away from equipment vibrations for approximately 30 minutes to give them time to adjust to their environment. Remember too that differences in cell densities can lead to variations in cellular maturity.

Once plated, primary neurons should be left alone as much as possible, disturbing them only for media changes. Any sort of shaking or movement can prevent your neurons from growing long-term, causing variability in your culture data.

Finally, when conducting your next experimental application such as neurotoxicity, gene expression or drug discovery efforts keep in mind types of reagents and substrates to improve your overall neuroscience studies.

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