CYAGEN B28 INTRODUCTION: COMPARED WITH B27

INTRODUCTION

B28 Neuron Culture Supplement (50X, Cat. No. BNCS-50101) is an optimized serum substitute developed to support growth of neurons from the CNS. It is used together with ***Neuron Basal Medium*** (Cat. No. GXXNR-03011) and another additive, L-Glutamine, at a final concentration of 0.5 mM.

B28 Neuron Culture Supplement (50X) is also a serum-free stocking solution for the long-term viability of neurons (derived from the hippocampus and other brain-derived tissues). It can be added to Cyagen Neuron Basal Medium or NEUROBASAL™ and NEUROBASAL™ -A media for the culturing of neurons. This product contains BSA.

Features

1. Supports optimal growth and long-term survival of neurons.
2. Excellent ability to promote adhesion of cells and the formation of visible networks.
3. Compatible with Cyagen Neuron Basal Medium or Invitrogen NeuroBasal™ medium.
4. Reduces glial cells in culture.
5. Can be used for most CNS neurons, especially neurons of embryonic origin from rats and mice.
6. Provides batch-to-batch consistency.

METHODS

Poly-L-Lysine/Laminin coating of tissue culture plate

1. The day before plating cells, prepare the coated plates with PLL/laminin.
2. Dilute Poly-L-Lysine (PLL) stock solution (1mg/mL) with water to yield a 15μg/mL solution.
3. Add enough PLL solution into the culture vessel to completely cover the base.
4. Swirl until PLL solution coats the entire base. Allow it to sit for at least 30 minutes at room temperature.
5. Aspirate off all of the PLL solution and rinse the vessel once with sterile water. Aspirate after rinsing.
6. Using sterile 1X PBS, dilute the Laminin stock solution (1mg/mL) to a final concentration of 15μg/mL.
7. Add enough Laminin solution into the culture vessel to completely cover its base. Incubate overnight at 4°C. Coated vessels can be stored in the Laminin solution at 4°C for up to one week.
8. Just before use, aspirate the Laminin solution from the coated vessel and wash the wells once with 1X PBS. Aspirate after rinsing.

Media (Cyagen Neuron Basal Medium and NEUROBASAL™)

1. Add 1X B28 Supplement (Cyagen, B28 Neuron Culture Supplement, Cat. No. BNCS-50101) in NEUROBASAL™ medium and 0.5 mM L-glutamine.
2. Add 1X B27 Supplement (Gibco) in NEUROBASALT medium and 0.5 mM L-glutamine.

Neurons

Primary hippocampal neurons are isolated from day-18.5 rat embryos. ***(Sprague-Dawley Fetal Rat Hippocampus Neurons,*** Cat.No. SHCFN-00001)

Plate at a cell density of 5000 cells/cm2 (isolated primary cells) and 1x105 cells/cm2 viable cells
(trawed cells) on a PLL/Laminin coated plate. Gently rock the culture vessel to evenly distribute the cells.

Change the cell culture medium after 24 hours. Subsequently, change the medium every 3-5 days.

RESULTS

Figure 1. 24 hours after plating the cells in the coated dish, neurons attach and form axons and dendrites. B28 (A-C) performs better than B27 (D-G).

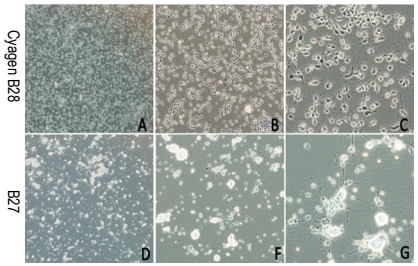


Figure 2. 48 hours after plating the cells in the coated dish, neurons cultured in B27 begin to form cell masses (D-G).

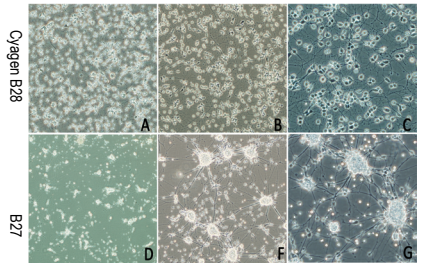
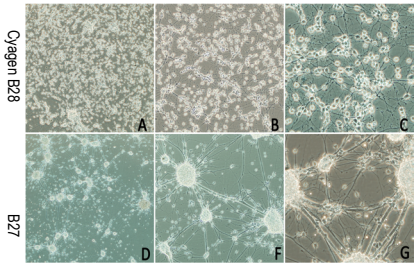


Figure 3. 72 hours after plating the cells in the coated dish, neurons begin to form numerous cell masses. Non-viable neurons are found within these masses.



A-C, Neurons cultured in Cyagen B28 supplement; D-G Neurons cultured in Gibco B27 supplement. A and D are 40X; B and F are 100X; C and G are 200X.

Figure 4. Neurons were cultured in B28 and B27 for 15 days. Neurons cultured in B28 have more viable cells and form more axons and dendrites. All pictures are 200X

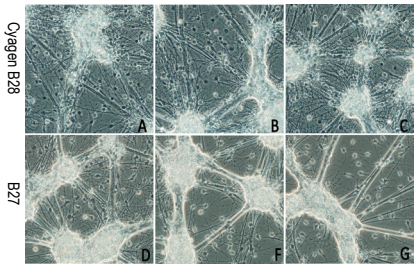
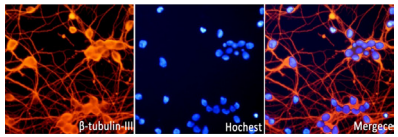


Figure 5. Neurons cultured in B28 expressing β-tubulin-III at 200X.



CONCLUSIONS

- B28 strengthens the neuron attachment.
- B28 prevents the neurons from forming cell masses and reduces the number of non-viable cells.
-Neurons that are cultured long-term in B28 have greater numbers of viable cells, and tend to form more axons and dendrites.

REFERENCES

Greory J Brewer, and John R Torricelli. (2007) Isolation and culture of adult neurons and neurospheres. ***Nature*** 2: 1490-1498.

CHENGSONG XIE, William R, Markesbery, and Mark A, Lovell. (2000) Survival of Hippocampal and Cortical Neurons in a Mixture of MEM and B27 Supplemented Neurobasal Medium. ***Free Radical Biology & Medicine*** 28: 665-672.

TECHNICAL SUPPORT

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Material Safety Data Sheets (MSDSs) are available upon request.

The Certificate of Analysis (COA), which provides detailed quality control information for each product, is also available at the Cyagen website.

Learn more about B28