B-27™ Plus Neuronal Culture System

PSC-Derived Neuron Applications

Catalog Numbers A3653401, A3582801 and A3582901

Pub. No. MAN0018046 **Rev.** 1.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Gibco™ B-27™ Plus Neuronal Culture System is comprised of B-27™ Plus Supplement (50X) and Neurobasal™ Plus Medium. This system represents an evolution of the neuronal cell culture products, B-27™ Supplement and Neurobasal™ Medium that are designed to provide maximum *in vitro* survival, improved maturation, and functionality of PSC-derived neurons. This user guide includes recommended protocol and an appendix with specific guidance for using the B-27™ Plus Neuronal Culture System with the most frequently used PSC-derived neuron models including monolayer NSC, rosette derived NSC, and factor-driven induced neurons "iN".

Contents and storage

Table 1 B-27™ Plus Neuronal Culture System, Cat. No. A3653401

Contents	Cat. No.	Amount	Storage	Shelf life[1]
Neurobasal™ Plus Medium	A3582901	500 mL	2°C to 8°C; Protect from light.	12 months
B-27™ Plus Supplement (50X)	A3582801	10 mL	–20°C to –5°C; Protect from light.	

[1] Shelf-Life duration is determined from Date of Manufacture.

Use

Maturation and maintenance of stem cell-derived neurons.

Note: For use with primary neuron applications, see *B-27™ Plus Neuronal Culture System User Guide*, (Publication Number MAN0017319).

Before you begin

Prepare 200mM ascorbic acid

Dissolve 1 g ascorbic acid 2-phosphate sesquimagnesium salt hydrate in 17.3 mL distilled water, then filter through a 0.22 µm filter.

Note: If not using solution right away aliquot 100 µL–200 µL into sterile tubes, and store at –5°C to –20°C in the dark for up to 6 months.

Prepare B-27™ Plus Neuronal Maturation Medium

Thaw the frozen B-27™ Plus Supplement (50X) at room temperature for ~1 hr or overnight at 2°C to 8°C.

IMPORTANT! Do not thaw the frozen supplement at 37°C

Mix the following components:

Reagent	Volume
Neurobasal™ Plus Medium	96 mL
B-27™ Plus Supplement (50X)[1]	2 mL
GlutaMAX™ Supplement	1 mL
CultureOne™ Supplement (100X)[1]	1 mL
Ascorbic acid (200 mM)	100 µL

[1] Supplement can be thawed at room temperature for ~1 hr or 2°C to 8°C overnight, and then aliquoted and frozen at –5°C to –20°C to allow for the preparation of smaller volumes of complete medium. Avoid repeated thawing and freezing. Once thawed do not leave thawed supplement at 4°C for more than two weeks.

B-27™ Plus Neuronal Maturation Medium can be stored at 2°C–8°C in the dark for up to 2 weeks. Warm media in a 37°C water bath for 5–10 minutes before using.

Coat culture plates

Coat plates with Poly-D-Lysine

1. Dilute the Poly-D-Lysine solution in sterile DPBS to prepare a 50 µg/mL working solution.
2. Coat the surface of the culture vessel with the working solution of Poly-D-Lysine (e.g. 50 µL/well of a 96-well plate).
3. Incubate the vessel at room temperature for 1 hour.
4. Remove the Poly-D-Lysine solution and rinse culture surface 3 times with a large volume of distilled water (e.g. 100 µL/well of a 96-well plate).

   Make sure to rinse the culture vessel thoroughly as excess Poly-D-Lysine solution can be toxic to the cells.
5. Remove final distilled water rinse and leave the coated culture vessel uncovered in the laminar hood to dry.

   The culture surface will be fully dry after 2 hours.

   Plates can be used immediately or stored at 4°C. For storage at 4°C tightly wrap the vessel with Parafilm™ film and use within one week of coating.

Add laminin coating

1. Thaw a vial of laminin at room temperature.

   Note: Thawed laminin can be aliquoted and stored at −80°C. Avoid repeated thawing and freezing.
2. Dilute the thawed laminin solution to 3 µg/mL with sterile distilled water, to create a working solution.
3. Add laminin solution into Poly-D-Lysine coated culture vessel to cover the whole surface, and incubate in a 37°C, 5% CO_2 incubator for 1 hour.
4. Immediately prior to seeding cells aspirate laminin solution from coated culture vessel.

Culture neurons

1. Start with PSC or NSC population then differentiate cells toward a neural lineage.

   NSCs must be cultured in a neuronal differentiation medium for 3–7 days before switching to B-27™ Plus Neuronal Maturation Medium for neuronal maturation and maintenance (see Figure 1).

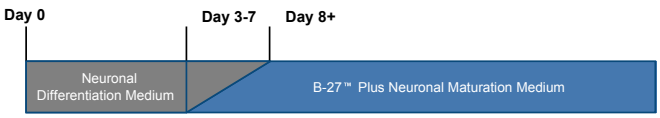

Figure 1 Culture NSCs for 3–7 days in Neuronal Differentiation Medium before transitioning to B-27™ Plus Neuronal Maturation Medium

Note: NSC differentiation medium is dependent on NSC derivation method [see "Guidelines for use of differentiation media and maturation media", page 3].

2. Once cells adopt a neuronal morphology (see Figure 2), remove half of spent medium and replace with equal volume of pre-warmed fresh complete B-27™ Plus Neuronal Maturation Medium.

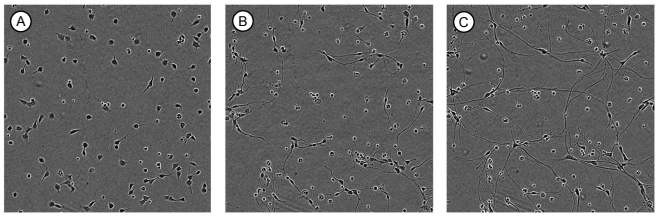

Figure 2 NSC differentiation and guidance on switching to B-27™ Plus Neuronal Maturation Medium

A, B) NSCs in the initial stages of neuronal differentiation. C) Cells ready to switch to B-27™ Plus Neuronal Maturation Medium. Cells have a neuronal-like morphology with neurites extending out and contacting neighboring cells.

3. Change spent medium every 3–4 days thereafter. (For high density cultures, change media every 2–3 days).

   When changing medium, remove half spent medium from each well and add the same volume of pre-warmed fresh B-27™ Plus Neuronal Maturation Medium into each well of plates and return them into a 37°C CO_2 incubator.
4. Maintain maturing neurons with B-27™ Plus Neuronal Maturation Medium for 3–10 weeks or longer depending on NSC lines and the purpose of experiments.

   Note: Differentiating neurons detach easily. When removing spent medium, do not touch cells with pipette tips. Also, add fresh medium gently toward the wall of culture plates.

Troubleshooting

Observation	Possible cause	Recommended action
Flat cells	Non-neural cells in starting NSC population	Check the purity of NSCs derived from hPSCs. A population which is >90% Sox1+ will reduce occurrence of non neural cell types.
		Treat cells with anti-mitotic compounds such as FUDR or AraC.
The media has turned yellow	Improved cell survival leads to greater media consumption.	Change media more frequently.
		Reduce seeding density of original number of cells.
	Serum or growth factors were added to the media	Don't add serum. B-27™ Plus Neuronal Culture System has been optimized to use without added serum or growth factors.
Cultures show clumped morphology	Problem with substrate coating	Reduce laminin coating concentration (recommend 1–3 µg/mL).
		Reduce seeding density of original number of cells.
		Check quality of Poly-D-Lysine coating.
		Adding laminin to refeed after >2 weeks may help.
	Non neural Cells displacing neurons from substrate	Check the purity of NSCs derived from hPSCs.
		Try increasing CultureOne™ Supplement concentration in the media

Appendix

Guidelines for use of differentiation media and maturation media

There are a variety of methods to derive NSCs from PSC lines. Depending on the derivation method, the NSC population may have varied requirements for efficient neuronal differentiation.

When starting with NSCs we recommend an NSC neuronal differentiation medium for 3–7 days. Once cells have adopted a neuronal-like morphology (see Figure 1), cultures should be transitioned to B-27™ Plus Neuronal Maturation Medium for continued maturation and maintenance of the neuronal cultures.

Methods to generate neural progenitors using NSC derived from either:

- Monolayer based differentiation such as (Neural Induction Medium)
- Neural Rosette formation

Here we provide recommended medium conditions for efficient neuronal differentiation of NSCs derived by the monolayer method or rosette formation. The conditions presented are guidance examples based on internal optimization using these PSC-derived neuron systems with the B-27™ Plus Neuronal Culture System. Different NSC derivation methods or cell lines may require some further optimization

Guidelines for differentiation

Prepare Monolayer Derived NSC Differentiation Medium

Mix the following components:

Reagent	Volume
Neurobasal™ Medium	95.9 mL
B-27™ Supplement[1]	2 mL
GlutaMAX™ Supplement	1 mL
CultureOne™ Supplement (100X)[1]	1 mL
Ascorbic acid (200 mM)	100 µL

[1] Supplement can be thawed at 2°C to 8°C overnight or quickly in a 37°C water bath for about 5 minutes, and then aliquoted and frozen at –5°C to –20°C to allow for the preparation of smaller volumes of complete medium. Avoid repeated thawing and freezing.

Prepare complete Rosette Derived NSC Differentiation Medium

Mix the following components:

Reagent	Volume
DMEM/F-12 Medium	86.9 mL
StemPro™ hESC Supplement[1]	2 mL
BSA (25%)	7.2 mL
GlutaMAX™ Supplement	2.5 mL
CultureOne™ Supplement (100X)[1]	1 mL
GDNF Recombinant Human Protein (20 µg/mL)	100 µL
BDNF Recombinant Human Protein (20 µg/mL)	100 µL
dcAMP (500mM)	100 µL
Ascorbic acid (200 mM)	100 µL

[1] Supplement can be thawed at 2°C to 8°C overnight or quickly in a 37°C water bath for about 5 minutes, and then aliquoted and frozen at –5°C to –20°C to allow for the preparation of smaller volumes of complete medium. Avoid repeated thawing and freezing.

Plate and differentiate NSCs

Differentiate NSCs

1. Dissociate expanded hPSC-derived NSCs in culture with StemPro™ Accutase™ Cell Dissociation Reagent or thaw frozen PSC-derived NSCs.
2. Resuspend dissociated or thawed NSCs with 5–10 mL DPBS, no calcium, no magnesium.
3. Centrifuge the cells at 300 × *g* for 5 minutes and aspirate the supernatant.
4. Resuspend NSCs in 1–2 mL of pre-warmed Neuronal Differentiation Medium.
5. Determine the concentration of viable cells using your preferred method.
6. Dilute the NSC suspension with pre-warmed Neuronal Differentiation Medium to an appropriate density.

   NSCs can be seeded at a density in the range of 2–10 × 10^4 cells/cm^2, for example for a 24-well plate 0.5 mls per well of a cell suspension of 1.6 × 10^5 cells/mL yields 4 × 10^4 cells/cm^2.
7. Aspirate the laminin solution from Poly-D-Lysine and laminin-coated plates immediately before plating cells.
8. Gently mix the tube containing NSCs and add an appropriate amount of diluted cell suspension into each well of culture plates.
9. Move the culture plates in several quick back-and-forth and side-to-side motions to disperse NSCs across the surface and place them gently in a 37°C CO_2 incubator.
10. At 2–3 days after NSC plating, if cells are not yet showing a neuronal morphology, remove half volume of spent medium and add same volume of pre-warmed fresh Neuronal Differentiation Medium into each well of plates and return them into a 37°C CO_2 incubator.

Neuronal maturation

1. 3–7 days after NSC plating once cells adopt a neuronal-like morphology, remove half volume of spent medium and add the same volume of pre-warmed fresh B-27™ Plus Neuronal Maturation Medium into each well of plates and return them into a 37°C CO_2 incubator.
2. Change spent medium every 3–4 days thereafter.

   When changing medium, remove half spent medium from each well and add the same volume of pre-warmed fresh B-27™ Plus Neuronal Maturation Medium into each well of plates and return them into a 37°C CO_2 incubator.

   Note: CultureOne™ Supplement is recommended to suppress continued and undesired NSC proliferation during neural differentiation and maturation. For additional details, see**CultureOne™ Supplement for Neuronal Cell Culture**.

   Note: The B-27™ Plus Neuronal Maturation Medium can also support improved functionality of factor-driven induced neuronal "iN" cells which typically utilize overexpression of lineage specific factors to rapidly induce neuronal cells. B-27™ Plus Neuronal Maturation Medium is recommended for use following initial induction steps (~ 1 week from onset of protocol), for neuronal maturation. There are many variations of protocols for generating induced neurons. Optimal timing should be empirically determined by user.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Neurobasal™ Medium	21103049
B-27™ Supplement (50X)	17504001
Neurobasal™ Plus Medium	A3582901
Nunc™ MicroWell™ 96-Well Microplates	167008
Poly-D-Lysine	A3890401
CultureOne™ Supplement (100X)	A3320201
UltraPure™ DNase/RNase-Free Distilled Water	10977015
GlutaMAX™ Supplement	35050061
DPBS, no calcium, no magnesium	14190250
DPBS, calcium, magnesium	14040117
Laminin Mouse Protein, Natural	23017015
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501
DMEM/F-12 Medium	11320033
StemPro™ hESC Supplement	A1050901
BSA (2%)	A1000801
GDNF Recombinant Human Protein	PHC7041
BDNF Recombinant Human Protein	PHC7074
cdAMP	Sigma-Aldrich, D0627
Ascorbic acid 2-phosphate sesquimagnesium salt hydrate	Sigma-Aldrich, A8960

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.